

CHEMICAL CONTROL OF NUTSEDGE
(*Cyperus rotundus* L.) AND THE
METABOLISM OF 3,6-DICHLORO-*O*-ANISIC
ACID (DICAMBA)

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INTRODUCTION

Nutsedge (Cyperus rotundus L.) is one of the most persistent weeds in crop lands. There has been much research directed toward eradication of nutsedge in cultivated lands. The best treatments from these studies gave only partial or temporary control.

At the beginning of this study there was no successful treatment economically feasible in large scale agriculture. Because of this weed's generally high tolerance to herbicides, it seemed desirable to test various fallow treatments when protection of a simultaneous crop would not be a problem.

The new herbicide 3,6-dichloro-p-anisic acid (dicamba) is promising against many weeds at low application rates. As the studies progressed, it became evident that dicamba was not effective against nutsedge. This suggested that nutsedge detoxified dicamba. There had been no studies of the detoxication of dicamba by nutsedge or any other higher plants. Dicamba is effective for weed control in field corn, but its approval has been withheld pending better information on its residues and metabolites. Metabolic studies of dicamba were undertaken in nutsedge and extended to corn and other species of higher plants.

REVIEW OF LITERATURE

Nutsedge and Its Control

Nutsedge is one of the most persistent weeds in croplands and is very often a serious pest in vegetable crops and in nursery stocks. This weed not only causes reduction in yield through competition for water and minerals in the soil, but also increases the cost of cultivation and lowers crop quality. It is also a problem in lawns, where it spoils the appearance of the turf. Bell et al. (1962) pointed out that nutsedge rhizomes grew through potato tubers, thus increasing the proportion of culls. Nutsedge tubers were also found to pass through bean-shelling equipment, necessitating hand sorting.

Although nutsedge is a native of Asia, it is distributed widely in all warm regions and is found in Africa, Australia, and in the warmer parts of Europe. The plant is adaptable to a wide variety of soils and environmental conditions in tropical or subtropical regions (Muenscher, 1955). In the United States, it is found from Virginia to Florida, Kansas, and Texas. Yellow nutsedge (Cyperus esculentus L.) is widespread in eastern North America, including Delaware, Massachusetts, Rhode Island, and New York.

Although usually called nutgrass, it is a perennial sedge belonging to the family Cyperaceae which has 72 genera, in which the genus Cyperus has about 700 species. The genus Cyperus belongs to the subfamily Scirpoideae (Lawrence, 1963). Of the species of the

genus Cyperus, Cyperus rotundus L., purple nutsedge, and Cyperus esculentus L., yellow nutsedge, are by far the most serious weeds.

The appearances of the yellow and the purple species are similar and the vegetative organs of the plants superficially resemble grasses. However, they can be distinguished easily from the true grasses by their triangular stems, closed leaf-sheaths, and three-ranked leaves.

Description of nutsedge plant

Leaves of purple nutsedge are bright green in color, linear, and have a deep furrow in the middle lengthwise. The tubers of purple nutsedge are larger than those of yellow nutsedge and grow in chains that develop in all directions from the mother plant. Purple nutsedge is slightly shorter than the other species. Georgia (1925) stated that these sedges are so named due to their dark purplish brown and pale yellow brown colored spikes, respectively. The tubers of purple nutsedge have a pungent, bitter taste compared to the almond-like flavor of the tubers of yellow nutsedge.

Ranade and Burns (1925) described the visible seed as really a fruit which had a triangular compressed hard-coated brown nut of minute size. The fruit is an achene which measures approximately 1/30th by 1/40th in.in size. Within this is the true seed, containing endosperm and a microscopic embryo. When the seed germinates the shoot of the early seedling consists of a mesocotyl, coleoptile attached to the first node and true leaves from successive nodes. A swelling occurs at the junction of the mesocotyl and the coleoptile. From this area develop adventitious roots and a basal bulb. Tubers develop at the ends of rhizomes which grow from the basal bulb. Basal bulbs are formed

within 2 weeks after the first foliar parts emerge from a tuber (Ranade and Burns, 1925; Justice, 1946).

Results indicated that fresh seeds, which are partially dormant, can be made to germinate by the application of ethylene chlorhydrin. It was found that nutsedge seeds were not killed by winter in north-eastern United States and could germinate during the following season. It has also been reported by Bell et al. (1962) that:

" . . . germination of nutsedge seed is favored by alternating daily temperatures of 85° to 95° F for 8 hours and 70° F for 16 hours. Seeds stored at room temperature or refrigerated for 3 years had a high percentage of viability. Alternating freezing and thawing or wetting and drying reduces the percent germination but does not kill all the seed."

Tubers

Rhizomes develop from both basal bulbs and tubers. The first tubers produced in a chain by an individual plant tend to form shoots but subsequent tubers tend to be dormant. Rhizomes are not reported to give rise to new growth except through tubers. Young rhizomes appear white and succulent, but as they grow older the endodermis walls thicken, and the cortical parenchyma and the epidermis disappear, leaving the rhizomes black and wiry. At an early stage the tubers are white; as they age the outer covering turns brown and finally black. Tuber size generally does not exceed 1 in. in length and 1.5 in. in width (Smith and Fick, 1937; Hauser, 1962a). Tubers store food for the other parts of the plant and are also a very effective means of propagation. New tubers are produced within 3 weeks after germination of an individual tuber (Andrews, 1960). Tuber formation is completed within 10 or 15 days after germination. Tubers

are provided with nodes, short internodes, buds, and scale leaves. The scale leaves are sloughed off at maturity (Ranade and Burns, 1925).

The number of shoots appearing above the soil is no indication of the number of tubers underground. Tubers are dormant when freshly harvested. Low temperatures and treatment with a solution containing thiourea (5%) or ethylene chlorhydrin (1%) can break the dormancy of tubers (Bundy et al., 1960). Andrews (1960) reported that at least 30% of field moisture capacity was needed for adequate germination. However, with the moisture content below 16%, the tubers generally died within 5 weeks. Dormant tubers sprouted at 68 F and a temperature of 80 to 97 F was ideal for subsequent growth. Tubers of both species of nutsedge have no specialized moisture retention mechanism, as do other fleshy organs like potato and onion bulbs (Day and Russel, 1955).

Dormancy of tubers

Tumbleson and Kommedahl (1963) observed that only 12% of the fall-harvested and 95% of the spring-harvested tubers in yellow nutsedge germinated, and indicated that the tubers passed through a period of dormancy. Low winter temperature broke the dormancy, as shown by the increased germination of fall-harvested tubers treated at 3 C. It appeared that there was some inhibitory substance present on the tuber epidermis which inhibited sprouting. This substance apparently was leached by rains, as indicated by the sevenfold increase in germination of washed fall-harvested tubers. It was also found that this tuber extract inhibited germination of washed tubers and six other crop seeds. The tuber extract was reported to be heat

stable, nonvolatile, water soluble, dialyzable, not adsorbed on charcoal, and could not be eluted from an alumina column by solvents.

Apical dominance

Apical dominance in potato tubers has been long recognized. In potato buds it was found that the apical bud prevented germination of lower buds (Michener, 1942). Muzik and Cruzado (1952) found a similar type of apical dominance in nutsedge tubers in which the first tuber in a chain inhibited the sprouting of the lower tubers. In a single tuber the apical bud sprouts first and suppresses the lateral buds from sprouting. The apical dominance is believed to be controlled by auxins. It was found that light induced sprouting of many buds, supposedly destroying the auxin effect. The dormancy of single tubers separated from the chain was broken. For this reason cultivation enhances rather than limits infestation of this weed. The plow breaks the chain and allows each single tuber to serve as a focal point for further infestation. The apical dominance of whole tubers also can be overcome by inverting the system or planting the system horizontally.

It is believed that natural or applied auxins move only in living tissues and are transported from tuber to tuber by rhizomes. This has been shown by various experiments. In a chain of tubers where rhizomes were killed by boiling water or acetone the tuber below the killed rhizome sprouted and remained unaffected by the application of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) above the killed portion. Tubers in a system with living rhizomes did not sprout when 2,4-D was applied to the top tuber only (Muzik and Cruzado, 1953).

It has been observed that a large majority of tubers occur in the upper 6 in. of soil, relatively few occur deeper than 8 in, and none are found deeper than 16 in. (Tumbleson and Kommedahl, 1961). These workers also reported that a single tuber could produce about 1,900 plants and nearly 8,900 tubers within a year on an area of about 34 sq ft on a silt loam soil. If only 1,280 tubers were distributed uniformly over an acre, 2.5 million plants and 1 ton of tubers could be produced within a year. Hauser (1962b) observed that during the first season tubers planted at 1-ft intervals produced approximately 3 million plants and 6.6 million tubers and bulbs in 1 acre of land. These results emphasize the capacity of nutsedge to infest or reinfest cultivated lands.

Eradication in the field

Many attempts had been made to control this pest in cultivated lands. Various methods were examined for control of this weed through cultivation and application of chemicals. Most of these methods either failed to control or resulted in partial control. The prolific abilities of this pest are such that partial control is valueless.

Cultural methods

Burgis (1951) stated that mechanical methods of eradication, such as plowing and fallowing, are so costly that they are hardly worthy of consideration. Day and Russel (1955) reported that tillage under dry conditions was highly effective in killing nutsedge tubers. Ranade and Burns (1925) observed that repeated deep plowing in order to expose the tubers to the sun would control a large percentage of the nutsedge. Growing a thick green manure crop and plowing it early in the dry season was effective in controlling nutsedge. Worsham

et al. (1964) indicated that nutsedge populations could be reduced by dense shading and repeated thorough tillage for several years. Pollock (1925) described such control methods as treatment with salts, arsenic compounds, fire, and smother crops. Various salts including sodium chlorate, calcium thiocyanate, and borates have been mentioned for this purpose (Anon., 1958; Worsham and Selman, 1966).

Biological control

Biological control of nutsedge has been attempted. Magalhaes and Franco (1962) reported that development of purple nutsedge was inhibited by an extract of bacterial nodules of Canavalia eusiformis roots, but the extract from the roots themselves showed no effect. Poiner (1964) reported that the best biological control was offered by the moth Bactra veruntana var. chrysea. The presence of egg and larval parasites of the moth decreased its effectiveness.

Tripathi (1964) found a beneficial lepidopterous larva feeding on nutsedge (C. rotundus) in jute fields. These caterpillars did not feed on the rhizomes. Rhodes (1965) mentioned C. rotundus as an important host of plant nematodes in Florida. None of these biological means have been reported to be significant in control of this sedge.

Fumigation

Fumigation eradicates living organisms in soil. Fumigation may kill all tubers in the soil if it reaches the deeper tubers. Leonard and Harris (1950) found 50 to 80% mortality of tubers when 2 lb of methyl bromide was applied per 100 sq ft. Day (1953) also reported methyl bromide to be an effective means for nutsedge control. Trichloronitromethane (chloropicrin) has been used as a fumigant against

nutsedge in some experiments (Martin, 1951). Thompson (1964) reported methyl bromide, ethylene dibromide, chloropicrin, sodium N-methyl-dithiocarbamate (SMDC), and 3,5-dimethyltetrahydro-1,2,5-2H-thiadiazine-2-thione (DMTT) were effective in destroying the tubers in soil.

Chemical control

Loustalot et al. (1953) reported 80 to 90% reduction of live tubers of nutsedge in plots plowed and then treated with 80 lb of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) per acre. Greenhouse experiments demonstrated that tubers planted 1 or 2 in. below the surface of monuron-treated soil were completely eradicated, and at a 4-in. depth the tubers were greatly reduced. At 6 to 12 in. the number of tubers had decreased after 5 1/2 months, but at a 15-in depth the number of tubers tripled. In untreated soil tubers increased many fold. Orsenigo and Smith (1953) stated that 20 lb or more of monuron per acre measurably retarded nutsedge. Bell and Bannister (1955) in a screening experiment indicated that monuron and other herbicides tested were not effective in eradicating yellow nutsedge. Kramer and Gregori (1962) found that 6 lb of monuron per acre failed to control C. rotundus. Angelini and Rumuer (1962) also reported that monuron failed to control nutsedge at 10 lb/A. Monuron, though offering some control of nutsedge at high rates, was not practicable for application to croplands.

Bell and Gardner (1962) noted that 5 lb of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) per acre did not control nutsedge and that the land became toxic to forage seedlings. Cole et al. (1960) reported complete control of nutsedge by 9 lb and adequate

control by 6 lb of atrazine per acre. Meade (1963) found 3 lb of atrazine suppressed yellow nutsedge for 3 weeks. Vengris (1963) found that 3 lb of atrazine was effective in controlling this weed in field corn throughout the growing season.

In preliminary studies of nutsedge control, Fertig (1961) found that rates of 2, 4, 6, or 8 lb of atrazine per acre were effective in reducing the stand of yellow nutsedge when applied as a post-emergence spray, and that among incorporated rates 6 or 8 lb/A were no more effective than 4 lb/A. Santelmann and Meade (1962) and Hargan et al. (1963) noted that 4 lb of atrazine per acre was necessary for the control of C. esculentus. Atrazine was not found to be useful in nutsedge control as 4 lb/A was very toxic to many crops without providing complete control.

The herbicide 2,4-D is used extensively as a selective weed killer, particularly among broadleaved weeds. Cowart and Ryker (1949) used 2 lb of the amine and ester forms of 2,4-D per acre and found reductions of 93 and 85% in nutsedge stand counts respectively. A combination of 164 lb sodium salt of trichloroacetic acid (TCA) with 2,4-D at 2 lb/A was more effective.

Experiments with 2,4-D as the amine, ester, or sodium salt in elimination of nutsedge gave partial to almost complete control for periods of from 3 weeks to 3 months (Anderson, 1958; Appalanaidu and Singh, 1962; Blackburn et al., 1952; Burgis, 1951; Christie, 1960; Loustalot, 1954; Marano, 1957; Rao et al., 1959; Rincon, 1961; Tippannavar et al., 1959; Vieitez, 1961; Worsham et al., 1964).

Temes and Jimerez (1961) in Spain indicated that a mixture of 1/2 lb of 2,4-D, 30 lb of sodium fluoride, and 5 lb of TCA per acre

applied 3 times at 20, 65, and 110 days after sprout emergence, destroyed both aerial and underground parts of C. rotundus with little residual toxicity in the soil. Hauser (1963b) demonstrated good control of nutsedge with repeated treatments of 2,4-D, the first being 1 or 2 weeks after shoot emergence on nonirrigated plots. Worsham et al. (1964) reported that repeated application of 2,4-D amine salt gave some control of nutsedge. Treating 3 or 4 times at 3 or 4-week intervals gave considerable control, but dormant tubers began to sprout thereafter. Thorough disking 1 week after application of 2,4-D greatly increased its effect.

The herbicide 3-amino-1,2,4-triazole (amitrole) was found to be translocated from foliage to roots in plants including nutsedge. Hauser (1954) and Anderson et al. (1964) demonstrated that amitrole readily penetrated through the foliage and was translocated into the tubers in Cyperus spp. Accumulation of this compound in growing points and in tubers was observed. Autoradiographs showed that amitrole followed the stream of photosynthate as observed in the movement of growth regulators. From similar autoradiograph studies with radioactive amitrole treatment on both species of nutsedge, Anderson et al. (1964), Donnally and Rahn (1961), and Hill et al. (1962, 1963b) indicated that C¹⁴ labelled amitrole was translocated upward and downward. Anderson suggested that the superiority of amitrole over 2,4-D and other compounds resulted from its ready translocation.

Hauser (1963a) found that nutsedge was susceptible to the repeated application of amitrole at 8 lb/A, beginning 4 weeks after initial emergence. Nutsedge appeared resistant to amitrole 6 weeks after emergence. Amitrole-T (amitrole activated with ammonium

thiocyanate) at 5 or 10 lb/A applied when C. rotundus was 2 to 7 in tall did not kill the underground tubers.

TCA and 2,2-dichloropropionic acid (dalapon) are excellent grass killers and have been frequently tested for control of nutsedge. Orsenigo and Smith (1953) noted effective control by the application of TCA prior to tuber formation of yellow nutsedge. Macedo (1961) reported 60% control of yellow nutsedge by 17 lb of dalapon per acre. Saidak (1961) studied translocation in nutsedge with C^{14} labelled dalapon and found that dalapon translocated freely in the plant system after root absorption, but found no accumulation in the parent tuber.

The herbicide S-ethyl N,N-di-n-propylthiolcarbamate (EPTC) is a promising herbicide for suppression of nutsedge tubers. EPTC has been tested very extensively, and to the best of our knowledge was the best available herbicide for control of nutsedge as our work commenced. Excellent control was obtained from 10 to 40 lb of EPTC per acre when the chemical was incorporated by tillage operation (Anon., 1959b). EPTC at 14 lb/A provided excellent control of nutsedge in Venezuela (Anon., 1959a). Antognini et al. (1959) observed that 3 or 4 lb of EPTC per acre did not kill the tubers but prevented them from germinating. Soil incorporated EPTC at 4 or 6 lb/A gave commercial control of this weed. Durfee et al. (1960) found that recovery of this weed was practically nil 1 year after treatment with 40 lb of EPTC per acre.

Ten pounds of EPTC per acre provided commercial control of nutsedge in potato and bean fields (Saidak, 1958; Sawyer et al., 1960). Nutsedge control averaged 94% 9 weeks after application with 6 lb of EPTC per acre (Trevett and Austin, 1959). Holt et al. (1962) found

that tubers germinated readily in soil treated with EPTC. They found that EPTC at 12 and 16 lb/A was very effective in killing nutsedge tubers. Repeated applications of EPTC were significantly better in terms of tubers killed than a single application at the same total rate of the herbicide. Thorough incorporation of the compound to a depth of 5 in by mechanical means was significantly more effective than incorporation by leaching.

Reports from the Tropical Pesticide Research Institute in Arusha, Tanganyika (Anon., 1961a) indicated that 5 lb of granular EPTC per acre gave limited control of C. rotundus. Hocombe and Ivens (1961) from that institute found that shoot density was reduced 75% after 36 weeks by 8 lb of granular EPTC under very dry conditions. Fertig (1961) found that 6 lb of EPTC per acre was more effective in reducing the stand of nutsedge than other herbicides tested. Donnally and Rahn (1961) demonstrated by autoradiographic studies that EPTC applied to soil did not enter ungerminated tubers of C. esculentus. In foliage applications, EPTC was translocated acropetally but not besipetally. Experiments elsewhere showed that EPTC killed the shoots and inhibited emergence for as long as 16 weeks but had little permanent effect on underground tubers. Tuber sprouting was not inhibited even at 16 lb of EPTC per acre (Cowart and Ryker, 1949; Holt et al., 1961; Horowitz and Gil, 1963; Santelmann and Meade, 1962). Green and Hocombe (1964) found from studies in East Africa that 4 lb of EPTC per acre incorporated at two 6-week intervals to a depth of 7 to 9 in. gave 85 to 88% control for 16 weeks. EPTC may not eradicate nutsedge completely but can be used very effectively in the field for suppressing tuber germination.

Sasser and Locascio (1966) reported that 12 lb of EPTC per acre offered good control of nutsedge with little or no injury to beans or cucumbers planted after 6 months.

Hauser and Parham (1966) observed that 4 lb of S-propyl N,N-dipropylthiocarbamate (vernolate) per acre placed 1.5 in. beneath the soil surface consistently controlled 90 to 100% of the nutsedge (C. rotundus). Depth of placement seemed critical. Control of nutsedge decreased with increase in the depth of placement from 1.5 to 5.5 in. In another study on the response of yellow nutsedge to thiocarbamates, Hauser et al. (1966) found that subsurface application of S-propyl N-butyl-N-ethylthiolcarbamate (pebulate) and vernolate gave better control of C. esculentus but caused more injury to peanuts than a surface application which was later incorporated into the soil. Hauser (1966, 1967) further indicated that vernolate injected at 2 to 4 in depths at the emergence stage controlled nutsedge with little injury to peanuts. Vernolate incorporated after surface application did not provide as effective nutsedge control as offered by subsurface injection.

Veatch (1958) obtained some success in controlling yellow nutsedge with 3 or 4 lb of 4,6-dinitro-o-sec-butylphenol (DNBP) per acre as a postemergence spray. Orsenigo (1961) indicated excellent prolonged control by application of 3 lb 2-ethylamino-4-isopropylamino-6-methylmercapto-s-triazine (ametryne) per acre. Anderson et al. (1964) reported that N-(beta-O,O-diisopropylidithiophosphorethyl) benzenesulfonamide (betasan) at 10 and 20 lb/A provided good control of nutsedge. Potassium azide injected about 1.5 in. deep at a rate of 100 lb/A as a band treatment to cotton at the time of planting

controlled nutsedge for about 4 weeks (Baker, 1966). Worsham and Selman (1966) reported that vernolate at 2 to 2.5 lb/A controlled nutsedge in soybeans and peanuts. Many other new herbicides, some of which are not yet released by the U.S.D.A., are also being tested at many locations for use against nutsedge.

Sasser and Locascio (1966) reported that N'-(3-trifluoromethyl-phenyl)-N,N-dimethylurea (fluometuron) and 3-tert-butyl-5-chloro-6-methyluracil (Dupont 732), N-isopropyl-alpha-chloroacetanilide (CP-31393), and 2-bromo-6-tert-butyl-N-methoxymethyl-O-acetotoluidide (CP-45592) provided inadequate control of nutsedge. According to these workers, 2,6-dichlorobenzonitrile (dichlobenil) and N-hydroxymethyl-2,6-dichlorothiolbenzamide (TH-073-H) incorporated at 10 to 20 lb/A gave excellent control and 5 lb/A gave fair control of nutsedge; but the residual effect was enough to injure beans and cucumber grown 6 months after herbicide application.

Benzoic Acid Metabolism

The metabolism of benzoic acids in animals would provide clues for the metabolism of these compounds in the plants. The unsubstituted benzoic acid metabolism in plants and animals would give information regarding the possible metabolism of chlorinated analogs in plants. Among the herbicidally active benzoic acid analogs, the metabolism of 3-amino-2,5-dichlorobenzoic acid (amiben), 2,5-dichloro-3-nitrobenzoic acid (dinoben) and 2,3,6-trichlorobenzoic acid (TBA) has been studied. The degradation in plants of 3,6-dichloro-o-anisic acid (dicamba), a relatively new herbicide, has received attention only very recently.

Benzoic acid itself was readily absorbed and metabolized by pea epicotyl tissue. About 75% of the absorbed radioactivity from C^{14} carboxyl-labelled benzoic acid did not accumulate in the tissue. Most of the accumulated compound appeared as benzoylaspartic acid (Andrea and Good, 1957). Klambt (1961) reported the formation of benzoylasparagine from C^{14} carboxyl-labelled benzoic acid. A considerable amount of radioactivity was conjugated to benzoylglucose. Six other unidentified metabolites were also noted. This worker (Klambt, 1962) also detected the hydroxylation of benzoic acid to salicylic acid and its glycosides in *Helianthus hypocotyl*. Pallas (1960) pointed out that benzoic acid may be translocated in plants without being metabolized.

Metabolism of two commercially available chlorine-substituted benzoic acids, amiben and TBA, has been studied to a limited extent. Working with squash and cucumber, Baker and Warren (1962) showed that oxidative breakdown of amiben took place mainly in the roots.

Chlorobenzoic acids were found to be extremely persistent in plants and soils. Audus (1963) speculated that possibly these compounds were not easily broken down by enzyme systems common in plants. Substitution of amino- and nitro-groups in the 3 position on the ring enhanced selectivity in these compounds.

Colby et al. (1964) found that soybeans formed a conjugate of amiben from which free amiben could be released by alkaline hydrolysis. In his recent studies on the fate of amiben in soybeans, barley, and pigweed, Colby (1966) isolated the conjugate as the N-glucoside of amiben. Similar observations were also reported by Hodgson and his co-workers (1966). They stated that at least 90%

of the amiben in the roots was present as the glucoside and roots contained more than 90% of the amiben recovered from soybeans and other plants. Colby (1965) suggested that the formation of N-glucosylamiben in plants was a detoxication mechanism. No other metabolites were reported by these workers.

In studying the metabolism of 2,5-dichloro-3-nitrobenzoic acid (dinoben) in soybeans, Colby (1966) characterized the only metabolite of dinoben as N-glucosylamiben. On acid hydrolysis this conjugate yielded amiben. This indicated that the aromatic nitro group in dinoben was first reduced to the amine before being N-glucosylated as observed in amiben metabolism.

Dewey et al. (1962) and Phillips (1959) found the herbicide TBA to be very stable in the soil. Balayannis et al. (1965) described TBA as a very persistent herbicide in wheat plants. They found that this compound passed unchanged through the alimentary tract of the rabbit and the mouse. Earlier works by Williams (1949) and Park and Williams (1958) demonstrated that many benzoic acids, including monochloro derivatives, when fed to animals were excreted in the urine either unchanged or in the form of a simple conjugate. These benzoic acids were found to be detoxified by condensation with glycine to form hippuric acids. Balayannis et al. (1965), reviewing older works of Williams (1949), stated that when large amounts of the benzoic acids were fed to animals, a temporary exhaustion of the glycine supply resulted. Glucuronic condensation then became dominant and benzoyl glucuronide was excreted in the urine.

Dicamba is a very promising selective herbicide for control of green smartweed (Polygonum scabrum, Moench) (Friesen, 1962), wild

buckwheat (Fagopyrum convolvulus L.) (Key, 1962), and Tartary buckwheat (Fagopyrum tartaricum L. Gaertn) in wheat (Vanden Born, 1961). Fertig and Furrer (1963) reported dicamba to be a very effective weed killer in bluegrass. Skoglund and Coupland (1963) mentioned that dicamba was about 12 times more effective than 2,4-dichlorophenoxyacetic acid (2,4-D) for control of wild buckwheat.

The selectivity of dicamba in grain crops may be due to the presence of the methoxyl group. Broadhurst et al. (1966) stated that this selectivity is largely lost when the methoxyl group is replaced by a chlorine atom, as in the herbicide 2,3,6-trichlorobenzoic acid.

In general, dealkylation is one of the metabolic pathways of aromatic acids. Scheline (1966a), working with rat caecal contents (mixed with an incubation medium), showed that vanillic acid, isovanillic acid, 3-O-methylgallic acid, and syringic acid were demethylated to some extent. Using o-anisic, m-anisic, anisic, and veratric acids, which are not phenolic, he detected phenolic metabolites only from veratric acid. Veratric acid was demethylated to a small extent at both the meta- and para-positions as traces of both vanillic and isovanillic acids were found.

Working with microsomal fractions from rabbit and guinea pig livers, Axelrod (1956) found that certain alkyl aryl ethers were cleaved to phenols and aldehydes. This reaction required molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate as cofactors. In studies on selective demethylation, Tsukamoto et al. (1964) demonstrated that in brucine the m-methoxy group (with reference to the lactam nitrogen) was demethylated predominantly over the

p-methoxy. However, in both 4-acetamino and 4-nitroveratrole whose nitrogens have opposite substituent orienting effects, demethylation was predominant in the p-position. This led them to conclude that the occurrence of selective demethylation in vivo was not due to the electronic configuration but to other unknown factors. In residue analysis of dicamba in crops and milk, Smith et al. (1965) speculated 3,6-dichlorosalicylic acid (DCSA) might be a metabolite of dicamba.

Broadhurst et al. (1966) detected a demethylated product as a minor metabolite of dicamba in wheat and bluegrass which they identified as DCSA. They also isolated 5-hydroxy-3,6-dichloro-o-anisic acid as the major metabolite which accounted for 90% of the total applied dicamba. Conjugated dicamba was also detected as another minor metabolite in both these species. They found no detectable free dicamba in wheat plants after 18 days and only a small amount as a conjugate after 29 days.

Decarboxylation of 2,4-D in plant tissue has been reported by Bach and Fellig (1959, 1961), Cunney and Markus (1960), Weintraub et al. (1952), and many others. Tompsett (1958) indicated that gallic acid was decarboxylated to pyrogallol in human urine. Pyrogallol was also detected in the urine of rats by Booth et al. (1959) when they injected gallic acid intraperitoneally. Protocatechuic acid was also found to be decarboxylated in rat faecal and caecal extracts by Booth and Williams (1963). Scheline (1966b) observed that oral protocatechuic and gallic acid were decarboxylated by rats. He suggested that the formation of urinary catechol, pyrogallol, and resorcinol was due to the decarboxylation of dietary components. Recently Vanden Born and Chang (1967) detected labelled CO₂ from

Canada thistle treated with carboxyl-labelled dicamba. Broadhurst et al. (1966), however, did not find appreciable radioactivity in CO₂ trapped from wheat plants treated with C¹⁴ carboxyl labelled dicamba, which indicated negligible decarboxylation.

Evans (1956) and Rogoff (1961) reviewed the metabolism of phenoxyaliphatic acids and related aromatic compounds and indicated that 2,4-D was degraded through 2,4-dichlorophenol and 3,5-dichlorocatechol to alpha-chloromuconic acid. These reviewers also reported that a large group of aromatic compounds were converted to catechol or protocatechuic acid. The catechol was further degraded to cis-cis muconic acid and then to gamma-muconolactone. Protocatechuic acid was converted to a similar gamma-lactone through beta-carboxymuconic acid. Gaunt and Evans (1961) reported similar metabolic conversion of the closely related compound 4-chloro-2-methylphenoxyacetic acid (MCPA) in an unnamed gram-negative soil bacterium. MCPA was metabolized through the suspected 6-hydroxy-4-chloro-2-methylphenoxyacetic acid to 5-chloro-3-methylcatechol, thence to alpha-methyl-gamma-chloromuconic acid and finally to alpha-methyl-gamma-carboxymethylene- Δ^{α} -butenolide. It is possible that the substituted benzoic acids also undergo similar degradation in plants.

In a controlled dissipation study, the dimethylamine salt of dicamba was applied to growing oats. Residues in the oats declined from 16 ppm at 1 day to about 1 ppm at 7 days following application. No residue of dicamba was found in threshed grain from oats which were treated with 1 lb of dicamba under normal field conditions prior to or at the early boot stage (Anon., 1961b).

Working with autoradiographic techniques in grapes, Leonard et al. (1965) found no accumulation of dicamba in the roots. There was an accumulation at the margin of the leaves with concentration in the areas of the hydathodes. Vanden Born and Chang (1967) observed in Canada thistle that dicamba applied to foliage tended to accumulate in young developing leaves in the shoot apex and in sprouts from creeping roots. There was little retention of dicamba in roots when applied to rooting medium. Magalhaes and Foy (1967) also reported that accumulation of dicamba was very poor in underground organs of nutsedge (C. rotundus L.)

Hurtt and Foy (1965) reported that Black Valentine beans (Phaseolus vulgaris L.) were able to excrete dicamba in detectable amounts following foliar application. Linder et al. (1964) also detected similar exudation of dicamba from the roots of bean plants (Phaseolus vulgaris, variety Pinto). They demonstrated this exudation from treated plants into the soil was sufficient to affect adjacent untreated plants. Orth (1965) noted that treated grains left in the soil after harvest released toxic residues. Foy and Hurtt (1967) in their recent studies demonstrated that transfer of dicamba occurred from the treated plants to the untreated plants through soil, sand, and several liquid culture media following treatment of donor shoots by various means. They also found that six substituted benzoic acid growth regulators exhibited excretion phenomena.

Hilton (1965) found that several ring substituted derivatives of benzoic acid, including dicamba, inhibited pantothenate biosynthesis in Escherichia coli by directly inhibiting the conversion of

ketopantoate to pantoate. Using an isolated mitochondrial fraction from cotyledons of etiolated Cucumis sativus L., Foy and Penner (1965) observed that dicamba and four other benzoic acid herbicides in the range of 10^{-5} to 10^{-3} M caused stepwise inhibition of oxygen consumption in the tricarboxylic acid cycle when succinate was the substrate. However, dicamba showed essentially no inhibition of alpha-ketoglutarate utilization at 10^{-3} M concentration.

MATERIALS AND METHODS

Field Experiments

Experiment 1

The experiments on nutsedge control were performed on Arredondo loamy fine sand at the Agricultural Experiment Station in Gainesville, Florida. Five hundred pounds of 0-10-20 analysis fertilizer per acre was applied and thoroughly disked on April 12, 1965. The experimental area was infested heavily with purple nutsedge about 4 in high by May, 1965. A randomized block design including 40 treatments in four replications was used. The plots were 20 ft long and 12 ft wide with alleys of 6 ft between ranges and 20 ft between blocks. The herbicides tested are listed in Table 1 by their common and chemical names.

Treatments were applied to the nutsedge plants by a carefully calibrated 4-gal knapsack sprayer having a pressure regulator in the discharge and an effective boom width of 6 ft. This sprayer applied 26 gal/A. Measured amounts of herbicides were thoroughly mixed with 2 gal of water in the sprayer, and it was charged with nitrogen at a pressure of 80 psi. The herbicides were incorporated to a depth of 6 in. with a rototiller immediately after application. The uncultivated checks and the plots receiving surface spray were not disturbed.

Irrigation was provided when needed in addition to the rainfall in order to maintain good growth conditions for nutsedge development.

Table 1. Herbicides used in nutsedge control.

Common name	Chemical name	Concentration of active ingredient
EPTC	<u>S</u> -ethyl <u>N,N</u> -dipropylthiocarbamate	8 lb/gal
Amitrole-T 2,4-D	3-amino-1,2,4-triazole NH_4CNS 2,4-dichlorophenoxyacetic acid (ethylhexyl ester)	2 lb/gal 4 lb/gal
Dicamba	3,6-dichloro- <u>o</u> -anisic acid	4 lb/gal
Dichlobenil	2,6-dichlorobenzonitrile	50% W.P.
TH-073-H	<u>N</u> -hydroxymethyl-2,6-dichlorothiolo- benzamide	50% W.P.
Terbacil	3- <u>tert</u> -butyl-5-chloro-6-methyluracil	80% W.P.
Dupont-733	3- <u>tert</u> -butyl-5-bromo-6-methyluracil	80% W.P.
ACS-93	2-methoxy-3,6-dichlorobenzyl acetate	4 lb/gal
CP-31675	6- <u>tert</u> -butyl-2-chloro- <u>o</u> -acetotoluidide	75% W.P.
Fluometuron	<u>N'</u> -(3-trifluoromethylphenyl)- <u>N,N</u> - dimethylurea	80% W.P.
Pyrichlor	2,3,5-trichloro-4-pyridinol	1.5 lb/gal
Prometryne	4,6-bis-isopropylamino-2-methylthio- 1,3,5-triazine	80% W.P.
60-CS-88	Special formulation of dicamba	1 lb/gal
CP-31393	<u>N</u> -isopropyl- <u>alpha</u> -chloroacetanilide	65% W.P.
CP-45592	6- <u>tert</u> -butyl-2-bromo- <u>N</u> -methoxymethyl- <u>o</u> -acetotoluidide	4 lb/gal
Picloram	4-amino-3,5,6-trichloropicolinic acid	2 lb/gal

Stand counts.---One month after treatment, nutsedge shoots were counted in two areas of 1 sq ft each from a representative section of each plot. These data were statistically analyzed. One year after treatment, similar stand counts of nutsedge plants were taken on June 19, 1966. These data were also analyzed statistically. Other counts of nutsedge stand were recorded at intervals from June, 1965, to June, 1966.

In July, 1966, a square foot of soil was dug in each plot to a depth of 6 in. and then to 12 in. To separate the tubers, the soil was sieved with a screen having 1/4 in. mesh. The tubers from the first 6 in. and the second 6 in. of soil were collected separately. The live tubers were counted. The tuber count data were statistically analyzed.

Residue bioassay.---In order to test the residual effects of the more promising herbicides, oats (Avena sativa L. var. Moregrain) were planted by a power-driven seed drill on October 21, 1965, on selected plots. The live oat seedlings were counted in a randomly selected area of 2 sq ft on November 27, 1965; January 7, 1966; and March 15, 1966.

Approximately 2 lb of oat shoots at seed set were harvested on April 26, 1966, from untreated areas and from the plots treated with dichlobenil. These samples of plant material were packed in insulated boxes containing dry ice and shipped to the Thompson-Hayward Chemical Company in Kansas City, Kansas, for herbicide analysis.

Oat seed heads were harvested from three rows, each 3 ft long. The heads were dried at 180 F for 3 days and threshed. The cleaned seeds were weighed and the yield data were analyzed statistically.

Samples of oat grain collected on May 12, 1966, from the untreated areas and from plots treated with dichlobenil were sent to the Thompson-Hayward laboratories for residue analysis.

Experiment 2

Another field experiment was conducted at the same location to repeat the test with the most promising herbicides from the previous experiment. The herbicides used were terbacil, ACS-93, amitrole-T, dichlobenil, and TH-073-H. A randomized block design of three replications was used. Plot and alley size were the same as in the previous experiment.

Herbicides were sprayed on October 5, 1965, and were incorporated into the soil by a rototiller immediately following treatment. Irrigation was provided whenever needed.

Nutsedge shoots were counted in the same manner as in the first experiment in an area of 2 sq ft. The stand count data were taken on November 8, 1965, and July 4, 1966.

Laboratory Experiment

Plant material

Seeds of corn and barley were germinated between two layers of cheesecloth, supported by a perforated aluminum plate placed over a polyethylene pan filled with aerated water. The ends of the cheesecloth were immersed in water, which kept the seeds moist by capillary action. Roots were excised after 5 to 7 days and incubated immediately. The species used were Coker 67 field corn (Zea mays L.) and barley (Hordeum vulgare L., var. Manchuria X Rabat).

Incubation

Erlenmeyer flasks (300 ml) were used for incubations. In order

to prevent microbial growth, 1 ml of tetracyclin stock solution containing 3 mg/ml and 1 ml of streptomycin stock solution containing 0.25 mg/ml were included in the incubations. Each flask was provided with 5 ml of 0.4 M phosphate buffer (pH 5.6). The treatment flasks were brought to 1×10^{-3} M potassium salt of dicamba (analytical grade). All flasks were brought to a final volume of 100 ml with distilled water. Approximately 10 g of roots were placed in the solution. The flasks were covered with tissue and placed on a shaker running at 60 cycles/min. Half of the twelve flasks were untreated. The incubation periods were 8 or 10 hr. After incubation the roots were removed and stored in a freezer (-20 C) until assayed (Wilcox et al., 1963).

In other experiments seeds or nutsedge tubers were germinated in aluminum bread pans (9" X 5") in sand. Seven to 10 days after germination, the soil in half of the bread pans was treated with 3 and 5 lb of methanolic dicamba per acre. Roots and shoots were harvested 10 to 12 days after treatment. The plant materials were stored in the freezer until analyzed.

The frozen plant parts were ground in methanol for 5 min in a Sorvall omnimixer, whose chamber was immersed in a bath of dry ice in methanol at -50 C or lower. The slurry was centrifuged at 17,500 rpm (37,000 X g) for 1/2 hr in a Sorvall refrigerated centrifuge.

The clear supernatant fractions of treated and untreated samples were collected in round-bottomed flasks. Extracts were evaporated to dryness by lyophilization. Dried materials were again dissolved in 3 to 4 ml of methanol and filtered through a sintered-glass filter funnel under vacuum. The samples were reduced to 1 ml under a stream of nitrogen.

Thin layer chromatography

The methanolic solutions were applied to 8 X 8 in thin layer plates coated with 800 μ to 1 mm of silica gel G (Brinkmann). The plates were developed in a solvent system of ethyl ether:ligroin:formic acid (50:50:2). Plates were dried and the silica gel between Rf 0.2 to 1.0 scraped off the plate.

The silica gel was eluted first with 4 to 6 ml of methanol and then with three 10-ml aliquots of acetone. The acetone fraction was evaporated to dryness and was redissolved by adding the methanolic fraction. The volume of this eluted solution was brought to 0.1 ml.

Gas chromatography

A Barber-Colman 10c gas chromatograph, fitted with a hydrogen flame ionization detector, was used throughout the studies. The liquid phase consisting of 1.5% SE-30 polyester was supported on 90 to 100 mesh Anakrom ABS (Analab Inc., Hamden, Connecticut). A glass column, 2 M X 4 mm i.d., was conditioned at 220 C for several days. The flash heater and the detector were maintained 25 C above the column temperature. The column was operated isothermally at various temperatures with flow rates of argon or nitrogen as tabulated.

Alkylation

These samples were treated with sufficient ethereal higher diazoalkane to retain a yellow color. Alkylation was further catalyzed by adding one drop (0.03 ml) of boron trifluoride (0.7%) for each 3 ml of reaction mixture. The reaction was allowed to run for 30 min. At the end of this time the excess diazoalkane was destroyed by bubbling carbon dioxide through the solution. These samples were again

adjusted to a volume of 0.1 ml before analyzing by gas chromatography. Each of the authentic compounds, dicamba, DCSA, 5-hydroxydicamba, and 3,6-dichlorogentisic acid (DCGA) (2.5 mg) was dissolved in 0.5 ml of methanol. The samples were alkylated with the same diazoalkane as the plant extract for use as standards in gas chromatography. DCSA and DCGA yielded the same derivatives as did dicamba and 5-hydroxydicamba respectively when fully methylated by means of diazomethane. Higher diazoalkanes, on the other hand, yielded discrete derivatives from these phenolic acids and their methyl ethers (Wilcox, 1966).

Dicamba, DCSA, 5-hydroxydicamba, and DCGA (10 mg) were each dissolved in 0.5 ml of methanol, developed by thin layer chromatography (TLC), and eluted as previously described. The methanolic eluates were made to 0.5 ml, alkylated with diazobutane, and analyzed by gas chromatography. The percentage of recovery from TLC separation was calculated from the peak heights.

Phenolate assay

To assay alkylation of the three possible phenolic metabolites of dicamba, a phenolate difference spectrum assay was used. A Perkin Elmer 202 UV spectrophotometer was used for this purpose. A 5-ml sample aliquot was added to 5 ml of 0.2 M phosphate buffer (pH 10.1) and read against a reference solution containing an identical aliquot of sample diluted with 5 ml of 0.2 M phosphate buffer (pH 7). A graphic method was used to measure the absorbance. A base line was drawn between the points at 278 $m\mu$ and 318 $m\mu$ on the spectrum for the compound DCSA. The height of the curve was then read at 303 $m\mu$ relative to the base line, giving the absorbance directly. Calibration curves were prepared in this manner for concentrations which were

equivalent to a range of from 0.1 to 10% unalkylated DCSA. Similar assays using appropriate wavelengths were used for the other acids as tabulated.

DCSA, 5-hydroxydicamba, and DCGA (10 mg) were dissolved in 0.5 ml of methanol and alkylated with diazoethane for 30, 45, or 60 min as described before. The solutions were then evaporated to dryness and brought to a volume of 10 ml with water. Values for the remaining unalkylated phenolic hydroxyl were read from calibration curves prepared in the same manner. The percent alkylation was calculated by difference (Wilcox, 1967).

Nutsedge plants connected by rhizomes were collected from flats planted for that purpose in the greenhouse. Pairs of tubers connected by rhizomes were planted in separate bread pans without breaking the rhizomes. One pan within each pair was treated 7 days after planting with 5 lb of 3-tert-butyl-5-chloro-6-methyluracil (terbacil) per acre by means of a small atomizer. Another pair of untreated pans were included as a control. A gap was maintained between the pans within the pairs to reduce contamination. Ten days after treatment the vegetative and underground parts of nutsedge plants were harvested separately from treated, indirectly treated, and control plants. These plant materials were analyzed by Dr. H. L. Pease of the Pesticide Residue Laboratories, Research Division, E. I. duPont de Nemours and Company. The residue analysis data are tabulated.

In a similar experiment 5 lb of dicamba per acre was applied to nutsedge plants growing in sand. The plant parts were separated as before, and the dicamba and possible metabolites purified as

previously described. The extracts from the vegetative organs were alkylated with diazo-n-butane, and the extracts from the underground organs were alkylated with diazo-n-propane. The samples were analyzed by gas chromatography, and compared with the appropriate derivatives of the authentic acids.

RESULTS

Chemical Control of Nutsedge

Experiment 1

Mean stand counts obtained 1 month and also 1 year after herbicide application are arranged by decreasing number of nutsedge shoots in Tables 2 and 3. The Duncan multiple range test on the arithmetic mean of those stand counts is tabulated.

Stand counts.--The number of nutsedge plants on the soil surface may not be a true index of nutsedge populations in the field. However, data on stand counts recorded for a period of 1 year would give a better perspective of infestation. Data showing the stand counts during a year are summarized in Table 4.

The number of tubers counted in a representative area in two different intervals of depth appear in Table 4. The arithmetic means of the number of tubers collected from 1 cu ft of soil with the appropriate Duncan's multiple range test are presented in Table 5. Most of the tubers were in the upper 6 in. of soil. A few were found at a depth of 9 or 10 in, but none were found below 12 in.

Two pounds of 2,4-D per acre, whether incorporated or not, did not control nutsedge as indicated by shoot and tuber counts. Most rates of amitrole-T alone or in combination with 2,4-D failed to control nutsedge. Eight pounds of amitrole-T per acre, either alone or in combination with 2 lb of 2,4-D per acre as a surface spray,

Table 2. Effects of herbicides on stand counts of nutsedge one month after application in experiment 1.

Treatment May 19, 1965	Rate lb/A	Mean per sq ft	Duncan multiple range test ^a		
			5%	1%	
Check, cult.	-	19.5	a	a	a
2,4-D	2 spray	17.0	ab	ab	ab
Prometryne	4 inc.	16.6	ab	ab	ab
Fluometuron	4 inc.	16.4	abc	abc	abc
Amitrole-T + 2,4-D	4 + 2 inc.	16.1	abc	abc	abc
ACS-93	1 inc.	16.0	abc	abc	abc
CP-31393	20 inc.	16.0	abc	abc	abc
60-CS-88	1 inc.	15.5	abc	abcd	abcd
Amitrole-T	4 inc.	13.8	abcd	abode	abode
2,4-D	2 inc.	13.4	bode	abcdef	abcdef
Amitrole-T	8 inc.	12.1	bodef	abcdefg	abcdefg
CP-31675	2.5 inc.	11.5	bodefg	bodefgh	bodefgh
ACS-93	4 inc.	11.4	bodefg	bodefgh	bodefgh
Amitrole-T + dicamba	4 + 5 inc.	10.5	cdefg	bodefghi	bodefghi

Table 2 (continued)

Treatment	Rate lb/A	Mean per sq ft	Duncan multiple range test ^a		
			5%	1%	
ACS-93	0.5 inc.	10.1	cdefg	bdefghi	
Check, uncult.	-	8.9	defgh	cdefghij	
CP-45592	5 inc.	8.0	defgh	defghijk	
Amitrole-T + 2,4-D	8 + 2 inc.	7.8	efghi	efghijkl	
Amitrole-T + 2,4-D	4 + 2 spray	7.0	fghij	efghijklm	
Picloram + amitrole-T	2 + 8 inc.	6.5	fghij	efghijklmn	
Dicamba	5 inc.	6.5	fghijk	efghijklmn	
CP-31675	5 + 4 inc.	6.4	fghijk	efghijklmn	
Dupont-733	10 inc.	6.3	fghijk	efghijklmno	
Pyriethlor	5 inc.	5.9	ghijkl	fghijklmno	
Amitrole-T + 2,4-D	8 + 2 spray	5.9	ghijkl	fghijklmno	
CP-31675	5 inc.	5.8	ghijklm	ghijklmno	
Amitrole-T	8 spray	4.0	hijklmn	hijklmno	
Dichlobenil	5 inc.	3.1	hijklmn	ijklmno	

Table 2 (continued)

Treatment	Rate	Mean	Duncan multiple range test ^a	
			5%	1%
May 19, 1965	lb/A	per sq ft		
Dichlobenil	2.5 inc.	2.6	hijklmn	ijklmno
Dichlobenil	10 inc.	1.6	ijklmn	ijklmno
CP-31675	10 inc.	1.4	ijklmn	klmno
Terbacil	10 inc.	1.1	klmn	klmno
Dichlobenil + CP-31675	5 + 5 inc.	0.9	klmn	klmno
TH-073-H	5 inc.	0.6	lmn	lmno
Amitrole-T + dichlobenil	4 + 10 inc.	0.3	mn	mno
TH-073-H	10 inc.	0.1	n	mno
EFTC	8 inc.	0.1	n	mno
Dichlobenil	20 inc.	0.0	n	no
TH-073-H	20 inc.	0.0	n	no
Amitrole-T + TH-073-H	4 + 10 inc.	0.0	n	o

^a Difference in means followed by the same letter is not significant at the indicated level.

Table 2. Effects of herbicides on stand counts of nutsedge one year after application in experiment 1.

Treatment	Rate	Mean per sq ft	Duncan multiple range test ^a	
			5%	1%
ACS-93	1 inc.	21.7	a	a
60-CS-88	1 inc.	20.4	ab	ab
ACS-93	4 inc.	20.0	abc	abc
2,4-D	2 inc.	18.9	abcd	abcd
Amitrole-T + 2,4-D	4 + 2 inc.	18.1	abode	abcd
Prometryne	4 inc.	17.1	abodef	abcd
Amitrole-T + 2,4-D	8 + 2 inc.	16.7	abodefg	abode
ACS-93	0.5 inc.	15.9	abodefgh	abodef
CP-31675	2.5 inc.	15.7	abodefghi	abodef
Amitrole-T + 2,4-D	4 + 2 spray	15.0	bodefghij	abodef
CP-45592	5 inc.	15.0	bodefghij	abodef
Fluometuron	4 inc.	14.9	bodefghij	abodef
Amitrole-T	4 inc.	14.7	bodefghij	abodef
Pyrichlor	5 inc.	14.1	cdefghij	abodef

Table 2 (continued)

Treatment	Rate lb/A	Mean per sq ft	Duncan multiple range test ^a	
			5%	1%
May 19, 1965				
Picloram + amitrole-T	2 + 8 inc.	13.2	defghijk	bcdef
CP-31675 + amitrole-T	5 + 4 inc.	13.1	defghijk	bcdef
CP-31675	5 inc.	13.1	defghijk	bcdef
Check, cult.	-	12.9	defghijk	bcdef
CP-31393	20 inc.	12.5	efghijk	bcdef
CP-31675	10 inc.	12.0	efghijk	odef
2, 4-D	2 spray	11.1	efghijkl	defgh
Amitrole-T	8 inc.	11.0	fghijkl	defgh
Check, uncult.	-	10.9	ghijkl	defgh
Amitrole-T + dicamba	4 + 5 inc.	10.1	hijkl	efgh
Dicamba	5 inc.	9.6	ijklm	efghi
Amitrole-T + 2, 4-D	8 + 2 spray	9.2	jklm	efghij
Amitrole-T	8 spray	8.4	klmn	fghijk
EFTC	8 inc.	8.2	klmn	fghijkl

Table 2 (continued)

Treatment	Rate lb/A	Mean per sq ft	Duncan multiple range test ^a	
			5%	1%
Dichlobenil	2.5 inc.	5.6	lmno	ghijklm
Dupont-733	10 inc.	3.6	mnp	hijklm
Dichlobenil	5 inc.	2.7	nop	ijklm
Dichlobenil + CP-31675	5 + 5 inc.	1.7	nop	jklm
Dichlobenil	10 inc.	1.6	op	klm
TH-073-H	5 inc.	1.3	op	lm
Terbacil	10 inc.	0.9	op	m
TH-073-H	10 inc.	0.5	op	m
Amitrole-T + dichlobenil	4 + 10 inc.	0.4	op	m
Dichlobenil	20 inc.	0.1	p	m
TH-073-H	20 inc.	0.0	p	m
Amitrole-T + TH-073-H	4 + 10 inc.	0.0	p	m

^aDifference in means followed by the same letter is not significant at the indicated level.

Table 4. Effects of herbicides on the stand counts and number of tubers during a period of one year after application in experiment 1.

Treatment	Rate	Stand counts per sq ft					Tuber count per		Total counts
							sq ft (depth)		
		June 16 1965	Sept. 9 1965	Oct. 9 1965	Mar. 25 1966	June 19 1966	0-6"	6-12"	
May 19, 1965	1b/A								
ACS-93	1 inc.	8.0	21.0	21.5	20.5	21.5	110.2	25.7	135.9
60-CS-88	1 inc.	15.0	23.0	18.5	17.5	17.0	88.2	15.5	103.7
ACS-93	4 inc.	9.0	17.0	16.5	15.0	16.0	106.2	31.0	137.2
2,4-D	2 inc.	13.4	19.5	19.0	17.5	18.9	130.0	35.0	165.0
Amitrole-T + 2,4-D	4 + 2 inc.	16.0	22.5	17.0	18.0	18.0	113.0	30.7	143.7
Prometryne	4 inc.	16.0	17.0	18.0	16.5	17.0	75.0	19.2	94.2
Amitrole-T + 2,4-D	8 + 2 inc.	7.5	13.0	11.0	13.5	16.5	62.2	29.2	91.4
ACS-93	0.5 inc.	10.0	18.5	18.0	16.5	16.0	95.2	30.2	125.4
CP-31675	2.5 inc.	11.5	14.5	18.0	11.5	18.0	105.0	30.0	135.0
Amitrole-T + 2,4-D	4 + 2 spray	7.0	16.0	15.5	14.5	13.5	75.0	22.5	97.5
CP-45592	5 inc.	9.5	14.5	15.0	15.0	15.0	78.2	18.7	96.9
Fluometuron	4 inc.	16.5	20.0	17.5	19.0	15.0	115.7	22.5	138.2

Table 4 (continued)

Treatment	Rate	Stand counts per sq ft						Tuber count per		Total counts
		June 16 1965	Sept. 9 1965	Oct. 9 1965	Mar. 25 1966	June 19 1966	sq ft (depth)			
							0-6"	6-12"		
May 19, 1965	1b/A									per cu ft
Amitrole-T	4 inc.	13.0	21.0	17.0	16.5	14.5	63.0	23.5	86.5	
Pyrethlor	5 inc.	6.0	37.0	17.5	12.0	15.0	72.7	35.2	107.9	
Picloram + amitrole-T	2 + 8 inc.	6.5	13.5	8.0	9.5	13.0	88.7	44.5	133.2	
CP-31675 + amitrole-T	5 + 4 inc.	6.5	16.5	14.0	15.0	12.5	104.5	28.2	132.7	
CP-31675	5 inc.	9.5	17.0	13.0	15.5	13.0	66.0	19.2	85.2	
Check, cult.	-	7.0	19.5	17.5	20.5	13.0	122.5	27.2	149.7	
CP-31393	20 inc.	16.0	13.5	12.0	14.5	9.0	85.2	25.2	110.4	
CP-31675	10 inc.	1.5	14.5	12.0	11.0	10.5	66.5	29.2	95.7	
2,4-D	2 spray	17.0	11.5	9.5	15.5	11.1	62.2	31.7	93.9	
Amitrole-T	8 inc.	12.0	14.0	14.0	14.0	5.5	79.0	19.7	98.7	
Check, uncult.	-	9.0	10.0	11.5	11.5	11.0	91.2	11.7	102.9	

Table 4 (continued)

Treatment	Rate	Stand counts per sq ft						Tuber count per		Total counts per cu ft
		June 16 1965	Sept. 9 1965	Oct. 9 1965	Mar. 25 1966	June 19 1966	sq ft (depth)			
							0-6"	6-12"		
May 19, 1965	1b/A									
Amitrole-T + dicamba	4 + 5 inc.	10.5	17.5	20.0	11.0	10.0	76.0	25.7	101.7	
Dicamba	5 inc.	6.5	12.0	14.5	11.0	8.5	53.0	26.2	79.2	
Amitrole-T + 2,4-D	8 + 2 spray	6.0	18.5	12.0	12.5	5.5	65.2	27.2	95.0	
Amitrole-T	8 spray	4.0	11.0	6.5	5.5	8.5	23.2	6.7	29.9	
EPTC	8 inc.	0.0	16.5	10.5	8.5	9.0	55.0	20.0	75.0	
Dichlobenil	2.5 inc.	2.5	8.5	5.5	2.5	5.5	38.2	6.5	44.7	
Dupont-733	10 inc.	8.0	6.5	5.0	6.5	3.5	32.0	9.7	41.7	
Dichlobenil	5 inc.	3.0	5.0	3.5	3.0	2.5	44.0	10.2	54.2	
Dichlobenil + CP-31675	5 + 5 inc.	1.0	4.0	3.5	2.0	1.5	4.5	3.5	8.0	
Dichlobenil	10 inc.	1.5	4.5	1.5	3.0	1.5	7.7	2.0	9.7	
TH-073-H	5 inc.	0.5	4.5	3.5	0.5	1.5	7.0	2.2	9.2	
Terbacil	10 inc.	1.0	0.0	0.5	0.5	1.5	7.7	2.7	10.4	

Table 4 (continued)

Treatment	Rate	Stand counts per sq ft					Tuber count per		Total counts per cu ft
		June 16	Sept. 9	Oct. 9	Mar. 25	June 19	sq ft (depth)	6-12"	
		1965	1965	1965	1966	1966	0-6"		
May 19, 1965	1b/A								
TH-073-H	10 inc.	0.0	0.0	0.0	0.5	0.5	3.0	2.7	5.7
Amitrole-T + dichlobenil	4 + 10 inc.	0.0	2.0	0.5	1.5	0.5	4.7	3.5	8.2
Dichlobenil	20 inc.	0.0	2.0	0.25	0.5	0.0	29.5	4.0	33.5
TH-073-H	20 inc.	0.0	0.0	0.0	0.0	0.0	2.2	0.7	2.9
Amitrole-T + TH-073-H	4 + 10 inc.	0.0	0.0	0.0	0.0	0.0	0.7	0.2	0.9

Table 5. Effects of herbicides on nutsedge tuber counts one year after application in experiment 1.

Treatment	Rate lb/A	Mean per cu ft	Duncan multiple range test ^a	
			5%	1%
2,4-D	2 inc.	165.0	a	a
Check, cult.	-	149.7	a	ab
Amitrole-T + 2,4-D	4 + 2 inc.	143.7	ab	abc
Fluometuron	4 inc.	138.2	abc	abcd
ACS-93	4 inc.	137.2	abc	abcd
ACS-93	1 inc.	135.9	abc	abcd
CP-31675	2.5 inc.	135.0	abc	abcd
Picloram + amitrole-T	2 + 8 inc.	133.2	abc	abcd
CP-31675 + amitrole-T	5 + 4 inc.	132.7	abc	abcd
ACS-93	0.5 inc.	125.4	abcd	abcd
CP-31393	20 inc.	110.4	abcde	abcde
Pyrichlor	5 inc.	107.9	abcde	abcde
60-CS-88	1 inc.	103.7	abcde	abcdef
Check, uncult.	-	102.9	abcde	abcdef

Table 5 (continued)

Treatment	Rate lb/A	Mean per cu ft	Duncan multiple range test ^a	
			5%	1%
Amitrole-T + dicamba	4 + 5 inc.	101.7	abcde	abcdef
Amitrole-T	8 inc.	98.7	abcde	abcdefg
Amitrole-T + 2,4-D	4 + 2 spray	97.5	abcde	abcdefg
CP-45592	5 inc.	96.9	abcde	abcdefg
CP-31675	10 inc.	95.7	abcdef	abcdefg
Amitrole-T + 2,4-D	8 + 2 spray	95.0	abcdef	abcdefg
Prometryne	4 inc.	94.2	abcdef	abcdefg
2,4-D	2 spray	93.9	abcdef	abcdefg
Amitrole-T + 2,4-D	8 + 2 inc.	91.4	abcdef	abcdefg
Amitrole-T	4 inc.	86.5	abcdefg	abcdefg
CP-31675	5 inc.	85.2	abcdefg	abcdefg
Dicamba	5 inc.	79.2	abcdefgh	abcdefg
EPTC	8 inc.	75.0	abcdefghi	abcdefg
Dichlobenil	5 inc.	54.2	odefghi	abcdefg

Table 5 (continued)

Treatment	Rate lb/A	Mean per cu ft	Duncan multiple range test ^a	
			5%	1%
Dichlobenil	2.5 inc.	44.7	defghi	bcdefg
Dupont 733	10 inc.	41.7	defghi	bcdefg
Dichlobenil	20 inc.	33.5	efghi	cdefg
Amitrole-T	8 spray	29.9	efghi	defg
Terbacil	10 inc.	10.4	fghi	efg
Dichlobenil	10 inc.	9.7	ghi	efg
TH-073-H	5 inc.	9.2	ghi	efg
Amitrole-T + dichlobenil	4 + 10 inc.	8.2	ghi	efg
Dichlobenil + CP-31675	5 + 5 inc.	8.0	ghi	efg
TH-073-H	10 inc.	5.7	ghi	efg
TH-073-H	20 inc.	2.9	hi	fg
Amitrole-T + TH-073-H	4 + 10 inc.	0.9	i	g

^aDifference in means followed by the same letter is not significant at the indicated level.

offered 50 to 60% control of aerial portions of nutsedge for 1 year. However, tuber counts show no significant differences from those of the check plots. Amitrole-T in combination with dicamba, picloram, and CP-31675 offered poor initial control and practically no control 1 year after treatment. Nutsedge treated with amitrole-T showed chlorosis and gradually the aerial portions of the plants died. Most of the tubers underground were unaffected. New normal shoots emerged from treated tubers 3 or 4 months after application.

EPTC at 8 lb/A gave complete suppression for 1 month. The effect then declined rapidly. One year after application no significant differences in plant or tuber counts relative to the cultivated check existed.

Ten pounds of CP-31675 per acre controlled nutsedge very effectively for 1 month, but within 3 months the effects declined sharply and had disappeared at 1 year.

Terbacil or Dupont-733, each at 10 lb/A, provided excellent control of most weeds but did not prevent germination of nutsedge. The plots appeared to have a pure stand of nutsedge. After 3 to 4 days the newly emerged seedlings yellowed. They died within 7 to 10 days. New shoots emerged from the underground tubers, which also died within 10 days. Tubers were almost eradicated by Dupont-733. A total of only six tubers was obtained from three replications, but the fourth replication yielded a large number of tubers. This variation may have been due to improper incorporation of the herbicide. Terbacil gave identical results to Dupont-733 in destroying underground tubers. There was a highly significant difference between

the mean tuber count in the control plot and those from the plots treated with terbacil or Dupont-733.

Dichlobenil at 10 and 20 lb/A almost completely controlled nutsedge throughout the year. Statistically no significant difference was observed between these two rates in stand and tuber counts. Probably due to improper incorporation, a large number of tubers were harvested from the third replication treated with 20 lb of dichlobenil per acre, causing a high average tuber count. The other plots receiving this treatment were almost free of tubers and entirely free of aerial portions of nutsedge. Ten pounds of dichlobenil was equally effective. Although 5 lb of dichlobenil gave fair control of aerial nutsedge 1 year after application, this rate and 2.5 lb/A had little effect in eliminating tubers.

Mixtures of 5 lb of dichlobenil with 5 lb of CP-31675 and 10 lb of dichlobenil with 4 lb of amitrole-T per acre produced results identical to those from 10 lb of dichlobenil per acre as shown by plant and tuber counts. Plots treated with these mixtures of herbicides were free of aerial portions of nutsedge. These mixtures were slightly more effective than dichlobenil alone and significantly more effective than CP-31675 alone at the same rates.

Treatments of TH-073-H gave results similar to those of dichlobenil. Ten and 20 lb of TH-073-H per acre almost eradicated nutsedge tubers and no shoots were noted during the succeeding year. A mixture of 10 lb of the latter compound with 4 lb of amitrole-T per acre gave identical results. Treatment with 5 lb of TH-073-H controlled nutsedge less effectively than either 10 or 20 lb treatments. However, differences among these three rates were not significant.

Other herbicides listed in Table 4 were ineffective in controlling nutsedge. Significant differences were not noted among these treatments and the checks.

Residue bioassay.---The stand counts and the grain yield from the oat bioassay shown in Table 6 indicate the effect of possible residues of the herbicides on oats planted in those plots showing good control of nutsedge.

As no check plot was included in the experiment in the bioassay on oats, the plots treated with terbacil which produced an apparently normal crop were used as a standard for comparing the effects of other herbicides. The highest rates of dichlobenil and TH-073-H severely reduced the stand of oats. The surviving oats were stunted and chlorotic and produced extremely poor grain. Dichlobenil at 2.5 and 5 lb and TH-073-H at 5 lb/A caused no adverse effects on oat stands. Twenty pounds of TH-073-H and 10 and 20 lb of dichlobenil per acre indicated a significant decrease in yield at the 5% level from those produced by the other herbicides. The mixture of dichlobenil and CP-31675 caused limited plant stunting but grain yield was not affected. Ten pounds each of dichlobenil and TH-073-H caused early stunting and reduction in stands in the beginning, but the surviving plants recovered in later growth stages. Terbacil and Dupont-733 produced normal vigorous plants with considerably higher yields than other treatments. The grain yield was not significantly different from that produced by the two lower rates of dichlobenil or TH-073-H.

Each individual determination for three replications receiving the treatments of 2.5, 5, and 10 lb of dichlobenil per acre indicated

Table 6. Influence of herbicides on crop stand and grain yield of oats planted 5 months after application.

Treatment	Rate	Stand count			Grain yield	Duncan multiple range test ^a	
		Nov. 27	Jan. 7	Mar. 15		5%	1%
May 19, 1965	lb/A	1965	1966	1966	lb/A		
Dupont-733	10	40	18	18	1340	a	a
Terbacil	10	51	21	19	1330	a	a
TH-073-H	5	33	15	12	1020	ab	ab
TH-073-H	10	39	15	12	776	abc	abc
Dichlobenil + CP-31675	5 + 5	28	15	10	776	abc	abc
Dichlobenil	2.5	37	18	16	763	abc	abc
Dichlobenil	5	36	16	13	763	abc	abc
Amitrole-T + dichlobenil	4 + 10	31	13	11	715	bc	bc
Amitrole-T + TH-073-H	4 + 10	34	10	11	496	bc	bc
Dichlobenil	10	23	13	13	450	bc	bc
TH-073-H	20	14	8	8	280	c	c
Dichlobenil	20	27	11	12	275	c	c

^aDifference in means followed by the same letter is not significant at the indicated level.

no residue either of dichlobenil or its metabolite 2,6-dichlorobenzoic acid in either vegetative or ripe grain parts of oats.

Experiment 2

The control of nutsedge as indicated by the mean stand counts 1 month and 1 year after treatment along with the Duncan multiple range test in Experiment 2 are shown in Tables 7 and 8. The summarized data on stand counts of nutsedge as recorded during a period of 1 year appear in Table 9.

In this experiment, the herbicides terbacil, dichlobenil, TH-073-H, amitrole-T, and their mixtures which appeared promising for controlling nutsedge in the first experiment were tested again. The recently developed herbicide ACS-93 was also included.

Both rates of ACS-93 and the lowest rate of terbacil failed to control nutsedge. The number of plants of nutsedge appeared greater in the plots treated with the lower rate of terbacil than that in the check plots. ACS-93 at 4 lb/A provided excellent initial control for 3 months, but the effect had disappeared after 1 year. The lowest rates of dichlobenil and TH-073-H and the highest rate of terbacil gave only partial control of nutsedge. The highest rates of dichlobenil and TH-073-H, their combination, and their mixtures with amitrole-T provided perfect control throughout the year. The mixtures controlled nutsedge somewhat less effectively than the highest rates of the herbicides alone although there were no significant differences among these treatments.

Table 7. Effects of herbicides on stand counts of nutsedge one month after application in experiment 2.

Treatment Oct. 5, 1965	Rate lb/A	Mean stand per sq ft	Duncan multiple range test ^a	
			5%	1%
Terbacil	10	9.8		
Terbacil	5	7.8		
Check, cult.	-	7.0		
Amitrole-T	5	6.5		
ACS-93	2	1.8	a	a
Check, uncult.	-	1.7	ab	a
Dichlobenil	5	1.5	b	a
ACS-93	4	0	c	b
TH-073-H	5	0	c	b
TH-073-H	10	0	c	b
Dichlobenil	10	0	c	b
Amitrole-T + dichlobenil	5 + 10	0	c	b
Amitrole-T + TH-073-H	5 + 5	0	c	b
Dichlobenil + TH-073-H	5 + 5	0	c	b

^aDifference in means followed by the same letter is not significant at the indicated level.

Table 8. Effects of herbicides on stand counts of nutsedge one year after application in experiment 2.

Treatment Oct. 5, 1965	Rate lb/A	Mean stand count	Duncan multiple	
			range test ^a	
			5%	1%
Terbacil	5	35.5	a	a
Check, uncult.	-	25.5	a	a
ACS-93	2	24.5	a	a
Amitrole-T	5	24.0	a	a
Check, cult.	-	19.1	ab	ab
ACS-93	4	13.1	bc	bc
Dichlobenil	5	12.0	cd	bcd
Terbacil	10	9.1	cde	cde
TH-073-H	5	6.5	def	cdef
Amitrole-T + TH-073-H	5 + 5	3.7	efg	def
Amitrole-T + dichlobenil	5 + 10	1.7	fg	ef
Dichlobenil + TH-073-H	5 + 5	1.7	fg	ef
TH-073-H	10	0.5	g	f
Dichlobenil	10	0.1	g	f

^aDifference in means followed by the same letter is not significant at the indicated level.

Table 2. Effects of herbicides on stand counts of nutsedge during a period of one year in experiment 2.

Treatment	Rate	Nov. 8 1965	Dec. 6 1965	Jan. 5 1966	Mar. 15 1966	Apr. 27 1966	July 4 1966
Oct. 5, 1965	lb/A						
Terbacil	5	7.8	20.5	14.0	24.0	34.0	35.5
Check, uncult.	-	17.3	4.5	23.5	7.0	17.0	25.0
ACS-93	2	1.8	11.0	14.0	8.0	15.0	24.5
Amitrole-T	5	6.5	11.5	7.0	15.5	16.0	24.0
Check, cult.	-	7.0	20.5	17.5	11.0	18.5	19.0
ACS-93	4	0.0	1.6	0.5	5.5	9.5	13.0
Dichlobenil	5	1.5	0.5	0.0	3.5	7.0	12.0
Terbacil	10	8.1	13.5	6.5	22.0	22.5	9.0
TH-073-H	5	0.0	0.0	0.0	8.5	7.0	6.5
Amitrole-T + TH-073-H	5 + 5	0.0	0.0	0.0	0.5	2.0	3.5
Amitrole-T + dichlobenil	5 + 10	0.0	0.0	0.0	0.0	1.5	1.5
Dichlobenil + TH-073-H	5	0.0	0.0	0.0	0.0	1.0	1.5
TH-073-H	10	0.0	0.0	0.0	0.0	0.0	0.5
Dichlobenil	10	0.0	0.0	0.0	0.0	0.0	0.0

Metabolism of Dicamba and Translocation of Herbicides

The Rf values by thin layer chromatography of authentic standards of dicamba and the suspected metabolites are listed in Table 10. A mixture of dicamba, DCSA, 5-hydroxydicamba, and DCGA separated into two distinct bands--the upper band containing dicamba and DCSA and the lower band 5-hydroxydicamba and DCGA. Further separation within the paired unresolved compounds was not possible in this solvent system.

The retention times of the derivatives of standards and suspected plant metabolites alkylated with various diazoalkanes appear in Table 11. The threshold of detection of butyl derivatives of 5-hydroxydicamba and DCGA was 20 ng at 210 C and a flow rate of 109 ml/min. Dicamba and DCSA could be detected at a threshold of 50 ng when the column temperature was 175 C at the same flow rate. The threshold is defined as the amount of sample required to give a peak height twice the noise level.

In the gas chromatograph the alkylated derivatives of the standards separated in the order of dicamba, DCSA, 5-hydroxydicamba, and DCGA. As the chain length of the diazoalkane increases the volatility of the derivatives decreases, further separating the peaks of those derivatives from each other and the solvent peak on the chromatogram.

The peak corresponding to dicamba was detected in all the dicamba treated plant extracts. Dicamba was detected in the leaves of corn, barley, and nutsedge plants when application was made to the rooting medium. The peak corresponding to dicamba was found in root extracts of barley and corn when leaves were smeared with a methanolic solution of dicamba (0.5 mg per plant). Retention times of standards and

Table 10. Thin layer chromatography of dicamba and analogs.

Compound	Rf ^a
Dicamba	0.37
DCSA	0.40
5-hydroxydicamba	0.25
DCGA	0.26

^aSilica gel G in ether:ligroin:formic acid (50:50:2).

Table 11. Retention times of alkyl derivatives of dicamba and analogs.

Compound	Diazomethane	Diazoethane	Diazopropane	Diazobutane
	150 C ^a min	150 C ^a min	165 C ^b min	165 C ^a min
Dicamba	1.1	1.4	2.8	2.6
DCSA	1.1	1.7	4.0	3.7
5-hydroxy- dicamba	2.6	3.8	4.0	8.2
DCGA	2.6	4.5	16.5	13.3

^aArgon carrier at 170 ml per min, 22 psi drop.

^bArgon carrier at 120 ml per min, 18 psi drop.

extracts from dicamba-treated excised roots of corn and barley are presented in Table 12.

When extracts from dicamba-treated and untreated excised corn roots were alkylated with diazobutane and analyzed by gas chromatography three peaks appeared in the treated extracts which were not present in the controls. Two of these peaks corresponded to DCSA and 5-hydroxydicamba. The third peak found in the treated sample did not correspond to any of our four standards. The height of the peak corresponding to 5-hydroxydicamba from excised roots was large compared to the peak corresponding to DCSA. The excised roots of barley alkylated with diazobutane produced a peak corresponding to DCSA but no peak corresponding to 5-hydroxydicamba could be detected in this sample.

The retention times appearing in Tables 12 and 13 indicate that those peaks produced by the shoot and root extracts of barley treated with 3 lb of dicamba per acre agreed with those from standard DCSA, 5-hydroxydicamba, and DCGA. DCGA was also indicated in the root extracts of barley plants treated with 5 lb of dicamba per acre. The root extracts from the barley plants treated with 5 lb of dicamba per acre of 1 mg of dicamba per plant on the leaf showed peaks which differ slightly from the retention time of the authentic DCGA peak. In all cases the peaks corresponding to 5-hydroxydicamba and DCGA from the treated plant extracts were higher than the peaks corresponding to DCSA.

In the extracts from treated nutsedge shoots dicamba was detected from all the samples. All peaks other than that of dicamba did not

Table 12. Retention times of alkyl derivatives of standards and dicamba incubated corn and barley root extracts.

Sample	Diazopropane	Diazobutane
	165 C ^a min	170 C ^a min
Dicamba	2.6	2.6
DCSA	3.7	4.9
5-hydroxydicamba	7.9	12.0
DCGA	12.5	-
Treated excised corn root ^b		4.8
		11.9
		22.2
Treated excised barley root ^b		4.8
Barley roots ^b treated with dicamba at 3 lb/A	3.8	
	7.6	
	12.6	

^aArgon carrier at 170 ml per min, 22 psi drop.

^bThe peaks that appeared in treated extracts and not in untreated control.

Table 13. Retention times of alkyl derivatives of standards and dicamba incubated barley shoot and root extracts.^a

Sample	Diazopropane min
Dicamba	5.0
DCSA	5.4
5-hydroxydicamba	10.5
DCGA	15.5
Extracts of barley shoot incubated with dicamba at 3 lb/A	5.2
	10.2
	15.8
Extracts of barley root treated with dicamba at 5 lb/A	5.2
	16.3
Extracts of barley root from plants treated with 1 mg per plant on leaf	5.3
	15.6

^aAnalyses were made by F and M 400 gas chromatograph equipped with a flame ionization detector and packed with 8% SE 30 on 60-80 mesh chromosorb W and operated at 210 C with a flow rate of 60 ml/min.

correspond to any of the standards and were also present in the control extracts.

The recovery data for purification of the four acids by thin layer chromatography appear in Table 14. The recovery of these compounds ranged from 85% to 99%. The retention times of all the compounds added to blanks corresponded closely to the standards when alkylated with diazobutane.

Alkylation yields following catalyzed reaction periods of 30 min and 1 hr with diazoethane appear in Table 15. A reaction time of 30 min was enough to provide more than 99% alkylation of DCSA and 5-hydroxydicamba, while 1 hr was necessary for 95% alkylation of DCGA using diazoethane.

The retention times in Table 16 will indicate that dicamba was detected in shoot and tuber extracts of both directly and indirectly treated (i.e., from treated plants through connecting rhizomes) nutsedge plants. In the control samples from shoot extracts the peak corresponding to dicamba was absent. In the tuber extracts it was not possible to detect dicamba conclusively due to interfering substances.

The residue analyses of terbacil in nutsedge leaves and tubers which appear in Table 17 were obtained from Dr. H. L. Pease of the E. I. duPont de Nemours and Company. The threshold of detection of terbacil was 0.04 ppm by their method. A little over 6% of the terbacil detected in the treated shoots moved to the leaves of untreated plants through the connecting rhizomes. Very minute amounts of terbacil could be detected in the tubers of both directly and indirectly treated nutsedge plants.

Table 14. Percent recovery of the four acids from thin layer chromatography.

Compound	Percent recovery ^a
Dicamba	98.3
DCSA	86.7
5-hydroxydicamba	95.6
DCGA	84.6

^aAverage of four determinations.

Table 15. Phenolic alkylation yields from diazoethane catalyzed with 0.007% boron trifluoride.

Compound	Base line (m μ)	Peak height (m μ)	Percent alkylation	
			1/2 hr	1 hr
DCSA	278-318	303	99.9	-
5-hydroxydicamba	294-344	312	99.9	-
DCGA	225-301	266	89.0	94.8

^aPhenolate difference spectra method.

Table 16. Retention times of dicamba and dicamba treated
nutsedge shoot extracts.^a

Sample	Diazoethane
	135 C ^b min
Dicamba	2.8
Directly treated shoot extracts	2.8
Indirectly treated shoot extracts	2.8

^aTreated with 5 lb of dicamba per acre.

^bArgon carrier at 120 ml per min, 18 psi drop.

Table 17. Analyses of terbacil residue in nutsedge plants.^a

Sample	Rate	Residue
	lb/A	ppm
Tuber extracts		
Directly treated	5	1.40
Indirectly treated	0	0.05
Check	0	<0.04
Shoot extracts		
Directly treated	5	8.70
Indirectly treated	0	0.56
Check	0	0.17

^aThreshold of detection was 0.04 ppm. Analyses were made by Dr. H. L. Pease of Dupont by gas chromatography.

It was noted that symptoms of chlorosis appeared in the untreated nutsedge plants which were connected by a rhizome to terbacil-treated plants in a separate pot or pan. These observations were made repeatedly in greenhouse experiments.

DISCUSSION

Chemical Control of Nutsedge

Complete nutsedge eradication on an economic scale had not been achieved as this study commenced, although many herbicides had been tested. In this work, few of the herbicides showed very promising results in controlling nutsedge. A few other herbicides gave only partial control.

The herbicides EPTC and amitrole-T were being used by earlier workers with some success for control of nutsedge. In our experiment the effectiveness of EPTC was found to be only temporary. EPTC suppressed nutsedge for only 1 month. EPTC may be useful for fast-growing crops which cover the soil surface in a short time.

Amitrole-T gave very poor control of nutsedge. Mixtures of amitrole-T with dichlobenil or TH-073-H resulted in excellent control for 1 year. In Experiment 2, these mixtures did not give control superior to that obtained from dichlobenil or TH-073-H used alone at higher rates. However, a combination of dichlobenil or TH-073-H at 10 lb applied with 8 lb of amitrole-T per acre appeared to be very effective in controlling nutsedge. Although perfect control of nutsedge was effected by 20 lb of dichlobenil or TH-073-H per acre, the residual effect in the soil was sufficient to severely reduce the yield of oats planted 5 months later. The plots treated with these compounds at that high rate remained free from other weeds for a year or more. These chemicals could be used effectively in the

spring if cropping can be sacrificed for a period of a year or so. Otherwise, a fallow treatment in the fall with one of the effective herbicides would enable successful cropping in the next summer season, even though less effective than spring applications.

The recently developed herbicide TH-073-H has not been approved for commercial use, and it is understood that Thompson-Hayward Chemical Company has decided to suspend development of it. Although this new herbicide controlled nutsedge very effectively, the result was no better than that offered by dichlobenil at comparable rates.

Amitrole-T produced additive effects when applied in May, 1965, with dichlobenil or TH-073-H and the combinations controlled nutsedge better than the latter two compounds applied alone at the same rates.

Both dichlobenil and TH-073-H at the highest rates completely prevented germination of tubers and eventually killed those tubers in soil. In a greenhouse test inhibition of tuber sprouting was noticed when single tubers which had been soaked overnight in water were planted in flats and treated with these compounds at 10 lb/A.

Terbacil and Dupont-733 did not prevent germination of tubers. Tubers continued to germinate for a period of 1 month or so. Germinated plants became chlorotic in 3 to 4 days and always died within 10 to 12 days after emergence. During this time nutsedge populations appeared denser than those in the cultivated check. These two herbicides may have stimulated germination of the dormant tubers. This observation was repeated in a greenhouse experiment. Increased germination was noticed when single tubers were planted after being soaked for 1 hr in a solution of 2 mg terbacil in 2 liters of water. These unreplicated observations are not included in the results

section. Many herbicides kill the aerial portion of nutsedge but do not translocate from one tuber to another in the chains. Results in the greenhouse and analysis of residues indicated that terbacil translocated from plant to plant. The translocation of this herbicide would help kill untreated tubers connected by rhizomes to treated tubers.

It seems reasonable to incorporate these herbicides to a depth of 6 in or more in order to get the herbicides in contact with the tubers and to protect the herbicides from volatilization. When using less effective incorporation equipment such as discs, a second incorporation at right angles to the first would be helpful. Small plot size may prohibit this in research work.

The absence of any residue of dichlobenil or its metabolite in oats planted 5 months after application of 10 lb dichlobenil per acre suggested that this treatment may be useful as a fall fallow for a spring seeded crop or as an early spring fallow before a summer seeded crop.

Experiment 2 provides results confirming the effectiveness of dichlobenil and TH-073-H and their mixtures with amitrole-T. In this experiment, however, terbacil did not produce satisfactory control of nutsedge. This may be due to the time of application relative to the life cycle of the sedge. Hauser (1963a) noted that amitrole-T gave the best control of nutsedge when the plants were 1 month old. In the first experiment the plants were 4 to 6 weeks old and were probably more vulnerable to herbicides. In the second experiment the application was made when the plants were in late flowering stage. Within a month or two the tubers became dormant. It appears that the time of application of terbacil was critical.

Two applications, 3 to 4 months apart using a low rate, such as 5 lb of dichlobenil or terbacil plus 8 lb of amitrole-T per acre incorporated, might be a very effective means of nutsedge control.

Metabolism of Dicamba and Translocation of Herbicides

For the detection of minute amounts of metabolites of herbicides in plants a large amount of plant material is needed. One prepurification of metabolites by thin layer chromatography often resulted in a sample sufficiently pure for gas chromatography. In other cases it was preferable to repeat the clean-up procedure in order to eliminate interfering peaks arising from plant constituents. The Rf values of dicamba and DCSA were close, but it was possible to separate them on the thin layer plate as DCSA glowed under ultraviolet light but dicamba appeared as a dark spot. The other two standards, 5-hydroxydicamba and DCGA, had very close Rf values and fluoresce similarly under ultraviolet light, so that it was not possible to separate them in the solvent used in this work. Approximately 30 solvent systems were tested for separation of these two compounds without success.

Although it was not possible to purify all of the individual standards by thin layer chromatography, a mixture of these four compounds produced after alkylation with higher diazoalkanes well-separated, single, symmetrical peaks on the gas chromatograph. No interaction was noted when these four compounds were alkylated together. Thus in the analysis of these metabolites of dicamba, it was not necessary to purify the individual metabolites for gas chromatography.

Peaks from plant products having the same retention time as a standard resulting from one diazoalkane may be eliminated by the use of a different diazoalkane. Higher diazoalkanes have been found to be very useful for simultaneous gas chromatographic analysis of phenolic acids and their methyl ethers. Smith et al. (1965) noted that common derivatives were obtained from dicamba and DCSA when they were alkylated with diazomethane. Production of common derivatives may be expected from any phenolic acid and its methyl ether alkylated with diazomethane. Dicamba and 5-hydroxydicamba are the methyl ethers of DCSA and DCGA, respectively.

In alkylating these four compounds with diazomethane, dicamba and DCSA yielded a common derivative and 5-hydroxydicamba and DCGA yielded a common derivative. As predicted this difficulty did not appear when using higher diazoalkanes. A further advantage is the greater alkylating activities of the higher diazoalkanes relatively to diazomethane (Wilcox, 1967). In recent work where 5-hydroxydicamba was identified as a major metabolite of dicamba, derivatives were prepared by means of diazomethane. If there were any DCGA formed, it would have yielded the same derivative as 5-hydroxydicamba, i.e., methyl 3,6-dichloro-2,5-dimethoxybenzoate. This ambiguous derivative was used for the gas chromatography, infrared spectral, and mass spectral work upon which this identification was based (Broadhurst et al., 1966).

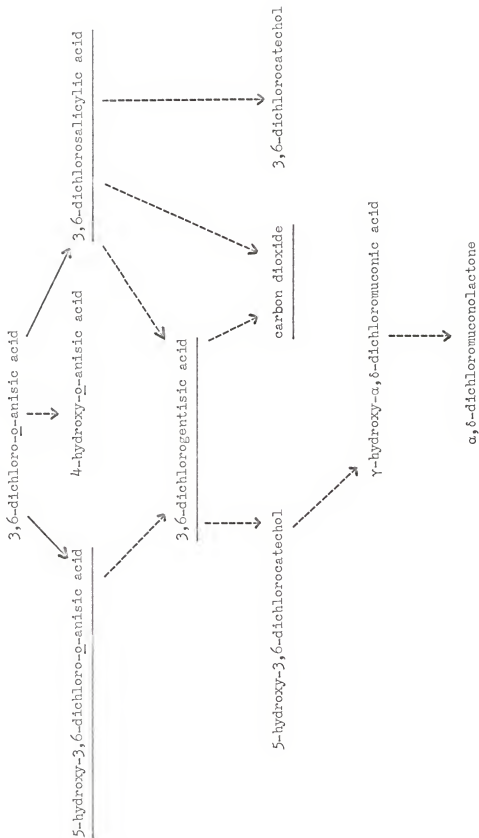
It was observed that a small percentage of the standards was lost in purification by thin layer chromatography. This loss would be repeated during further subsequent purification. At least three elutions with acetone were required for satisfactory recovery.

The derivatives prepared from dicamba, DCSA, 5-hydroxydicamba, and DCGA with different diazoalkanes were quite stable when stored in the freezer at -20 C. These derivatives could be detected without any apparent loss or decomposition 6 months after preparation.

Scheline (1966b) found that most nonphenolic methoxybenzoic acids including unsubstituted o-anisic acid, when incubated with caecal contents from rats, were not demethylated. The one exception was veratric acid which was demethylated to a limited extent. In studies of the metabolism of dicamba by Broadhurst et al. (1966) and in the present work, it is observed that DCSA was a minor metabolite. If the identification of DCGA in this work is correct, the results are similar to Scheline's in that the hydroxymethoxyaromatic acid was O-demethylated more easily than the methoxyaromatic acid. It is speculated that this may be a general biochemical mechanism in that hydroxylation of a methyl aryl ether precedes O-demethylation. From studies on the metabolism of dicamba and other aromatic acids, speculation leads to suggested sequences in the metabolism of dicamba, which appear on the next page. The solid lines indicate some evidence is available for the indicated reaction; dashed lines indicate pure speculation on the basis of other aromatic acids; where intermediates are underlined, there is some evidence for their production.

In this study dicamba was found to be metabolized primarily to 5-hydroxydicamba in excised corn roots. Evidence for the production of 5-hydroxydicamba in roots of treated barley plants was equivocal because a natural product having the same retention time was present in small amounts in roots of untreated plants.

Suggested sequences in the metabolism of dicamba



DCSA was found to be a minor metabolite in treated excised roots of corn and barley and in extracts from roots and shoots of treated intact barley plants. There was limited evidence for the production of DCGA in the roots and shoots of treated barley plants.

Evidence of rapid metabolism of dicamba in excised corn roots indicates that dicamba might be useful for weed control in corn. Relative freedom from residues would be expected, resulting in clearance for use by the United States Department of Agriculture.

SUMMARY

Field experiments were performed to find a herbicide suitable for providing complete eradication of nutsedge. For this purpose 17 different herbicides and some of their combinations were studied.

EPTC, amitrole-T, and CP-31675 initially gave good control of nutsedge, but the effect declined sharply with time. Dichlobenil at 2.5 or 5 lb/A did not give perfect control of nutsedge for 1 year but effected fair control for 1 year. Rates of 10 or 20 lb of dichlobenil per acre produced perfect control of nutsedge for 1 year but severely reduced the yield of oats planted 5 months after application. The highest rates completely eradicated tubers from the soil for 1 year. The analyses indicated no residues of dichlobenil or of its metabolite 2,6-dichlorobenzoic acid in vegetative parts and seeds of oats planted 5 months after treatment in the plots receiving 2.5, 5, and 10 lb/A.

The herbicide TH-073-H gave control of nutsedge identical to that produced by dichlobenil at their comparable rates. The highest rate of TH-073-H was also very toxic to oats and caused a poor grain yield of oats. The combinations of 8 lb of amitrole-T and 10 lb of dichlobenil or TH-073-H were just as effective in controlling nutsedge shoots and tubers as dichlobenil and TH-073-H applied alone. The mixture of dichlobenil plus CP-31675, each at 5 lb/A, gave excellent control of nutsedge shoots and tubers for 1 year.

Terbacil and Dupont-733 at 10 lb/A provided good control of nutsedge and almost eradicated tubers. Application of terbacil gave better results when nutsedge plants aged 4 to 6 weeks were treated than did treatment of the mature stage in the fall. External symptoms on nutsedge leaves and the residue analyses indicated that terbacil translocated from plant to plant through connecting rhizomes. All other herbicides tested, including dicamba, failed to control nutsedge.

In preliminary experiments procedures used in these studies did not detect any metabolites of dicamba from treated nutsedge. Excised roots of corn and barley were incubated with dicamba for 8 to 10 hr. The 7 or 10-day old barley plants were treated with 3 or 5 lb of dicamba per acre. Nutsedge plants germinated from tubers and grown for 7 or 10 days were treated with 5 lb/A. The plant parts were extracted in methanol. After eliminating most plant constituents through purification by thin layer chromatography, the eluates were alkylated with higher diazoalkanes. The samples were analyzed by gas chromatography and compared with authentic standards alkylated in the same manner.

DCSA was identified as minor metabolite in the extracts from excised roots of barley and corn. The major metabolite in extracts from excised corn roots was 5-hydroxydicamba. Limited evidence indicated that 5-hydroxydicamba and DCGA were the major metabolites of dicamba in extracts of roots and shoots of dicamba treated barley plants. Dicamba was detected in extracts of shoots of untreated nutsedge plants connected by a rhizome to a treated plant growing in a separate container. This indicated translocation of dicamba from plant to plant via rhizome.

BIBLIOGRAPHY

- Anderson, O. 1958. Studies on the absorption and translocation of amitrole (3-amino-1,2,4-triazole) by nutgrass (Cyperus rotundus L.). Weeds 6:370-385.
- Anderson, W. P., J. W. Whitworth, and J. McCaw. 1964. Chemical control of purple nutsedge Cyperus rotundus L. Res. Progress Rep., WWC.
- Andrea, W. H. and N. E. Good. 1957. Studies on 3-indoleacetic acid metabolism. IV Conjugation with aspartic acid and ammonia as processes in the metabolism of carboxylic acids. Plant Physiol. 32:566-572.
- Andrews, F. W. 1960. A study of nutgrass (Cyperus rotundus L.). Ann. Bot. 4:177-193.
- Angelini, A. and C. L. Rumuer. 1962. Un essai de lutte chimique contra Cypress rotundus (A trial to control C. rotundus chemically). Cotton et Fibres Trop. 17:357-360.
- Anon. 1958. Ecological, physiological, and edaphic aspects of weed control. Abstr. Meeting Weed Soc. Amer. 28-47.
- Anon. 1959a. El herbicida eptam resulta eficaz al corocillo (The weed killer eptam is effective against Cyperus rotundus). Not. Agr. Serv. Shell Agr. 2:41-42.
- Anon. 1959b. Hacia el control del corocillo mediante herbicidas (Chemical control of nutgrass). Not. Agr. Serv. Shell Agr. 32:3-4.
- Anon. 1961a. Progress Rep. Trop. Pesticides Res. Inst. 29:23-29.
- Anon. 1961b. Bull. No. 521-2. Velsicol Chemical Corporation.
- Antognini, J., D. F. Dye, G. F. Probandt, and R. Curtis. 1959. Control of quackgrass and nutgrass in horticultural and agronomic crops with eptam. Proc. NEWCC 13:50-51.
- Appalanaidu, B. and D. J. C. Singh. 1962. Studies on the control and eradication of nutgrass (Cyperus rotundus L.) by chemicals. Andhra Agr. J. 9:108-123.
- Audus, L. J. 1963. The physiology and biochemistry of herbicides. Academic Press, London.

- Axelrod, J. 1956. The enzymatic cleavage of aromatic ethers. *Biochem. J.* 63:634-639.
- Bach, M. K. and J. Fellig. 1959. The fate of C¹⁴-labeled 2,4-dichlorophenoxyacetic acid in bean stem sections. *Plant Physiol.* 34: (Suppl.) XVII-XVIII.
- Bach, M. K. and J. Fellig. 1961. Metabolism of carboxyl-¹⁴C-labelled 2,4-dichlorophenoxyacetic acid by bean stems: heterogeneity of the ethanol-soluble, ether-insoluble products. *Nature* 189:763.
- Baker, R. S. 1966. Special weed problems-nutsedge. Research Report. *Proc. SWC* 19:211.
- Baker, R. S. and G. F. Warren. 1962. Selective herbicidal action of amiben on cucumber and squash. *Weeds* 10:219-224.
- Balayannis, P. G., M. S. Smith, and R. L. Wain. 1965. Studies on plant growth-regulating substances. XIX. Stability of 2,3,6-trichlorobenzoic acid in wheat plants and in the rabbit and mouse. *Ann. Appl. Biol.* 55:149-157.
- Bell, R. S. and B. J. Bannister. 1955. Chemical control of northern nutgrass in potato fields. *Proc. NEWCC* 9:231-234.
- Bell, R. S. and P. B. Gardner. 1962. Experiments with control of nutgrass seed. *Proc. NEWCC* 16:73-75.
- Bell, R. S., W. H. Lachman, E. M. Rahn, and R. D. Sweet. 1962. Life history studies as related to weed control in the Northeast, 1-nutgrass. *Rhode Island Agr. Exp. Sta. Bull. No.* 364.
- Blackburn, F. H. B., D. M. Hanschell, and M. Clarke. 1952. Some aspects of weed control in Trinidad. *Trop. Agr.* 29:54-69.
- Booth, A. N., M. S. Masri, D. J. Robbins, O. H. Emerson, F. T. Jones, and F. DeEds. 1959. The metabolic fate of gallic acid and related compounds. *J. Biol. Chem.* 234:3014-3016.
- Booth, A. N. and R. T. Williams. 1963. Dehydroxylation of catechol acids by intestinal contents. *Biochem. J.* 88:66P-67P.
- Broadhurst, A., M. L. Montgomery, and V. H. Freed. 1966. Metabolism of 2-methoxy-3,6-dichlorobenzoic acid (dicamba) by wheat and bluegrass plants. *J. Agr. Food Chem.* 14:585-588.
- Bundy, O. M., W. F. Donnally, and E. M. Rahn. 1960. Growth and development of northern nutgrass as affected by certain environmental conditions. *Proc. NEWCC* 14:44.

- Burgis, D. S. 1951. Nutgrass control with 2,4-D. Proc. SWC 4: 24-25.
- Christie, G. A. 1960. Nutgrass. Cane Growers' Quart. Bull. 23: 116-117.
- Colby, S. R. 1965. Herbicide metabolism: N-glycoside of amiben isolated from soybean plants. Science 150:619-620.
- Colby, S. R. 1966. The mechanism of selectivity of amiben. Weeds 14:197-201.
- Colby, S. R., G. F. Warren, and R. S. Baker. 1964. Fate of amiben in tomato plants. J. Agr. Food. Chem. 12:320-321.
- Cole, R. H., C. D. Kesler, and F. B. Springer, Jr. 1960. Nutgrass control in field corn. Proc. NEWCC 16:316-320.
- Cowart, L. W. and T. C. Ryker. 1949. Studies on chemical control of Cyperus rotundus. Proc. SWC 2:61-62.
- Cunmy, M. J. and K. Markus. 1960. The breakdown of 2,4-dichlorophenoxyacetic acid in shoots and roots. Australian J. Biol. Sci. 13:486-500.
- Day, B. E. 1953. Soil fumigation with chlorobromopropene for the control of nutgrass. Hilgardia 21:593-605.
- Day, B. E. and R. C. Russel. 1955. The effect of drying on survival of nutgrass tubers. Calif. Agr. Exp. Sta. Bull. No. 751.
- Dewey, O. R., R. V. Lindsay, and G. S. Hartley. 1962. Biological destruction of 2,3,6-trichlorobenzoic acid in soil. Nature 195:1232.
- Donnelly, W. F. and E. M. Rahn. 1961. Translocation of amitrole, atrazine, dalapon, and EPTC in northern nutgrass. Proc. NEWCC 15:46.
- Dryden, R. D. 1964. Application of dicamba and mixtures of dicamba on Selkirk wheat. Res. Rep. Natl. Weed Comm., Western Canada Sec. 11:2-3.
- Durfee, J. W., W. H. Lachman, and W. C. Lincoln, Jr. 1960. Control of northern nutgrass with eptam and atrazine. Proc. NEWCC 14: 214-216.
- Evans, W. C. 1956. Biochemistry of the oxidative metabolism of aromatic compounds by microorganisms. Ann. Rep. Prog. Chem. (Chem. Soc. London) 53:274-294.

- Fertig, S. N. 1961. Preliminary results on the use of chemicals for nutgrass control in the field crops. Proc. NEWCC 15: 334-335.
- Fertig, S. N. and A. N. Furrer, Jr. 1963. Preliminary results on the control of dogfennel (Anthemis cotula) in winter wheat and winter barley. Proc. NEWCC 17:340.
- Foy, C. L. and W. Hurtt. 1967. Further studies on root exudation of exogenous growth regulators in Phaseolus vulgaris L. Abstr. Meeting Weed Soc. Amer. 40.
- Foy, C. L. and D. Penner. 1965. Effect of inhibitors and herbicides on tricarboxylic acid cycle substrate oxidation by isolated mitochondria. Weeds 13:226-232.
- Friesen, H. A. 1962. Green smartweed (Polygonum scabrum) control in Eagle oats with various herbicides. Res. Rep. Natl. Weed Comm., Western Canada Sec. 9:98-99.
- Gaunt, J. K. and W. C. Evans. 1961. Metabolism of 4-chloro-2-methylphenoxyacetic acid by a soil microorganism. Biochem. J. 79:25-26.
- Georgia, A. 1925. Manual of weeds. p. 68. Macmillan Co., New York.
- Green, D. H. and S. D. Hocombe. 1964. Experiments on the chemical control of Cyperus rotundus in East Africa. East African Conf. Soil-acting Herbicides, 3rd, Nairobi, p. 25.
- Greenham, C. G., and J. A. Robertson. 1963. A new approach to the use of 2,4-D esters for the control of nutgrass and other persistent weeds. Australian J. Exp. Agr. Anim. Husb. 3: 166-167.
- Hargan, R. P., G. W. Bayer, J. C. Ciolone, and R. O. Sweet. 1963. Some factors influencing nutgrass response chemicals. Proc. NEWCC 17:333-338.
- Hauser, E. W. 1954. Effects of 3-amino-1,2,4-triazole and derivatives on nutgrass and Johnson grass. J. Agr. Food Chem. 2: 680-681.
- Hauser, E. W. 1962a. Establishment of nutsedge from space-planted tubers. Weeds 10:209-212.
- Hauser, E. W. 1962b. Development of purple nutsedge under field condition. Weeds 10:315.
- Hauser, E. W. 1963a. Effects of amitrole on purple nutsedge at different growth stages. Weeds 11:181-183.

- Hauser, E. W. 1963b. Response of purple nutsedge to amitrole, 2,4-D, and EPTC. Weeds 11:251-252.
- Hauser, E. W. 1966. A promising method for control of nutsedge in peanuts. Proc. Natl. Peanut Conf. 4:82-84.
- Hauser, E. W. 1967. Res. Rep. SWC 20:45.
- Hauser, E. W., J. L. Butler, J. Shepherd, and S. A. Parham. 1966. Response of yellow nutsedge, Florida Pusley, and peanuts to thiocarbamate herbicides as affected by method of placement in soil. Weed Res. 6:338-345.
- Hauser, E. W. and S. A. Parham. 1966. Control of yellow nutsedge by thiocarbamate herbicides by placement in field soil. Abstr. Meeting Weed Soc. Amer. 9.
- Hauser, E. W. and J. T. Thompson. 1959. Rate of infestation of nutgrass (Cyperus rotundus L.) from space planted tubers. Weeds 12:173-179.
- Hill, E. R., W. H. Lachman, and D. N. Maynard. 1963a. Reproductive potential of yellow nutsedge by seed. Weeds 11:160-161.
- Hill, E. R., W. H. Lachman, and D. N. Maynard. 1963b. Translocation of amitrole in yellow nutsedge and its effect on seed formation. Weeds 11:165-166.
- Hill, E. R., W. H. Lachman, D. N. Maynard, and W. C. Lincoln. 1962. The effect of foliar application of amino triazole on the germination of northern nutgrass seed. Proc. NEWCC 10: 66-68.
- Hilton, J. L. 1965. Inhibition of pantothenate biosynthesis by substituted benzoic acids. Weeds 13:267-271.
- Hoccombe, S. D. 1962. Herbicide experiments in Arabica coffee. Res. Rep., Coffee Res. Sta., Lyamungu, Tanganyika, 39-41, 1961 (Weed Abstr. 11:1418).
- Hoccombe, S. D. and G. N. Ivens. 1961. Misc. Rep. Trop. Pest. Res. Inst., Arusha, Tanganyika, n.d.l., 282:15, 1961 (Weed Abstr. 10:1579).
- Hodgson, H. A., C. R. Swanson, and H. R. Swanson. 1966. The physiology of amiben glucoside formation. Abstr. Meeting Weed Soc. Amer. 37.
- Holt, E. C., W. W. Allen, and J. A. Lang. 1961. EPTC retards growth on nutgrass tubers. Crops and Soils 13:27.

- Holt, E. C., J. A. Lang, and W. W. Allen. 1962. The toxicity of EPTC on nutsedge. *Weeds* 10:103-105.
- Horowitz, M. and M. Gil. 1963. Experiments on the eradication of nutgrass (C. rotundus). *Hassadeh* 43 (12):1619-1622.
- Hurt, W. R. and C. L. Foy. 1965. Excretion of foliar applied dicamba and picloram from roots of Black Valentine beans grown in soil, sand and culture solution. (Abstr.) *Proc. NEWCC* 19: 602.
- Justice, O. L. 1946. Seed production, viability and dormancy in the nutgrass, Cyperus rotundus and Cyperus esculentus. *J. Agr. Res.* 73:303-318.
- Key, C. H. 1962. Comparison of Banvel compound for control of wild buckwheat Polygonum convolvulus. *Res. Rep. Natl. Weed Comm., Western Canada Sec.* 9:80-81.
- Klamt, H. D. 1961. Wachstumsinduktion und Wuchsstoffmetabolismus im Weizenkoleoptilzylinder. III. Stoffwechselprodukte der Naphthyl-1-Essigsäure und der Benzoesäure. *Planta* 56:618-631.
- Klamt, H. D. 1962. Conversion in plants of benzoic acid to salicylic acid and its β -D-glucosides. *Nature* 196:491.
- Kramer, M. and R. Gregori. 1962. Observacoes sobre a aplicacao de herbicidas em cafezais (Observation on the application of herbicides in coffee). *Sem. Brasil. Herb. Ervas Daninhas*, 184-194, 1960 (*Weed Abstr.* 11:742).
- Lawrence, G. H. M. 1963. *Taxonomy of vascular plants.* p. 393. Macmillan Co., New York.
- Leonard, O. A. and V. C. Harris. 1950. Nutgrass control with methyl bromide. *Proc. SWC* 3:132-134.
- Leonard, O. A., L. A. Linder, and R. K. Glenn. 1965. Absorption and translocation of herbicides by Thompson seedless (Sultavina) grape (Vitis vinifera L.). *Weed Res.* 6:37-49.
- Linder, P. J., J. W. Mitchell, and G. D. Freeman. 1964. Persistence and translocation of exogenous regulating compounds that exude from roots. *J. Agr. Food Chem.* 12:437-438.
- Loustalot, A. J. 1954. Studies on nutgrass (Cyperus rotundus L.) and its control. *Bull. Fed. Exp. Sta., U.S.D.A., Puerto Rico* 52:1-30.
- Loustalot, A. J., H. J. Cruzado, and T. J. Muzik. 1953. Effect of CMU on nutgrass (Cyperus rotundus L.). *Weeds* 2:196-201.

- Macedo, A. C. 1961. Accion del herbicida Dowpon en viveros de citricos (The action of the herbicide Dowpon in citrus nurseries). Turrialba 11:166.
- Magalhaes, A. C. and C. L. Foy. 1967. Aspects of the physiology of herbicidal action of dicamba in purple nutsedge (Cyperus rotundus L.). Abstr. Meeting Weed Soc. Amer. 44.
- Magalhaes, A. C. and C. M. Franco. 1962. Toxicidade de feijao de porco sobre a tiririca (Toxicity of sword beans for nutgrass). Bragantia 21:53-58.
- Marano, M. A. 1957. A spray for the destruction of nutgrass (Cyperus esculentus). Australian Patent, 1957, 206596 (Chem. Zentr. 130:16461) (Weed Abstr. 6:603).
- Martin, J. A. 1951. Nutgrass (Cyperus rotundus) can be killed with chloropicrin. Southern Seedsman 14(4):28-29.
- Meade, J. M. 1963. Yellow nutsedge killed by chemical. Crops and Soils 15:17.
- Michener, H. D. 1942. Dormancy and apical dominance in potato tubers. Amer. J. Bot. 29:558-568.
- Muenscher, W. C. 1955. Weeds, 2nd ed. Macmillan Co., New York.
- Muzik, T. J. and H. J. Cruzado. 1952. Structure and function in nutgrass, Cyperus rotundus. Proc. SWC 5:24.
- Muzik, T. J. and H. J. Cruzado. 1953. The effect of 2,4-D on sprout formation of Cyperus rotundus. Amer. J. Bot. 46:507-511.
- Orsenigo, J. R. 1961. Primary evaluation of preplanting and of pre and post emergence applied herbicides in vegetable and field crops. Mimeo Rep. Fla., Everglades Agr. Exp. Sta. 62:26.
- Orsenigo, J. R. and O. Smith. 1953. The chemical control of northern nutgrass, Cyperus esculentus L. Proc. NEWCC 7:329-339.
- Orth, H. 1965. The effects and after effects of 2-methoxy-3,6-dichlorobenzoic acid. Z. Pflanzenkrankh. Pflanzenschutz, Sonderh. No. 3:145-147 (Abstr. Chem. Abstr. 19, vol. 61).
- Pallas, J. E., Jr. 1960. Effects of temperature and humidity on foliar absorption and translocation of 2,4-dichlorophenoxy-acetic acid and benzoic acid. Plant Physiol. 35:575-580.
- Park, D. V. and R. T. Williams. 1958. The hydroxylation of foreign aromatic compounds in the animal body. Ann. Rep. Chem. Soc. 55:377.

- Phillips, W. M. 1959. Residual herbicidal activity of some chloro-substituted benzoic acids in soil. Weeds 7:284.
- Poiner, G. O. 1964. Studies on nutgrass insects in southern California and their effectiveness as biological control agents. J. Econ. Entomol. 57:379-383.
- Pollock, N. A. R. 1925. Eradication of nutgrass. Queensland Agr. J. 23:53-56.
- Ranade, S. K. and W. Burns. 1925. The eradication of Cyperus rotundus. Memoirs Dept. Agr. in India 13:99-181.
- Rao, A., J. A. Agarwal, and B. K. Kaushik. 1959. Effect of weedicides on nutgrass (Cyperus rotundus) shoots. Indian J. Agron. 4:48-49.
- Rhodes, H. L. 1965. Cyperus rotundus, an important host of plant nematodes in Florida. Plant Dis. Rep. 48:121:994-995.
- Rincon, D. J. 1961. Mediante el empleo de sustancias quimicas: control del corocillo o coquillo (Control of corocillo or coquillo (nutgrass) by chemicals). Ing. Agron. (Venezuela) 4:23-30, 1960 (Weed Abstr. 10:747).
- Rogoff, M. H. 1961. Oxidation of aromatic compounds by bacteria. Advan. Appl. Microbiol. 3:193.
- Saidak, W. J. 1958. Studies with EPTC for nutgrass control. Proc. NEWCC 12:196-198.
- Saidak, W. J. 1961. Translocation of dalapon in yellow nutsedge (Cyperus esculentus). Weeds 9:626-633.
- Santelmann, P. W. and J. A. Meade. 1962. The response of nutgrass to herbicides applied at varying stages of growth. Proc. NEWCC 16:321-326.
- Sasser, J. M. and S. J. Locascio. 1966. Effects of several herbicides on nutsedge control. Proc. SWC 19:254-260.
- Sawyer, R. L., S. L. Dallyn, W. H. Thorne, and G. H. Collin. 1960. Eptam for nutgrass control in potato. Proc. NEWCC 14:111-112.
- Scheline, R. K. 1966a. The decarboxylation of some phenolic acids by the rat. Acta Pharmacol. Toxicol. 24:275-285.
- Scheline, R. K. 1966b. Decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents. J. Pharm. Pharmacol. 18:664-689.
- Skoglund, N. A. and R. T. Coupland. 1963. Response of wild buckwheat to several post emergence herbicides. NEWCC Res. Rep. 20:24-25.

- Smith, E. V. and G. L. Fick. 1937. Nutgrass eradication studies. Relation of the life history of nutgrass, Cyperus rotundus L., to possible methods of control. J. Amer. Soc. Agron. 29: 1007-1013.
- Smith, M., H. Suzuki, and M. Malina. 1965. Analysis of dicamba in crops and milk including a rapid cleanup method. J. Assoc. Offic. Agr. Chemists 48:1164-1169.
- Temes, B. and C. Jimerez. 1961. Cyperus rotundus and its control with herbicides. Commun. Forest. Invest. Exp. (Madrid) 9:35.
- Thompson, J. T. 1964. Where are we in nutgrass control? Abstr. Meeting Weed Soc. Amer. 98-99.
- Tippannavar, M. B., S. V. Hiregondar, and H. R. Arakeri. 1959. Eradication of nutgrass (Cyperus rotundus). Mysore Agr. J. (India) 34:122-126.
- Tompsett, S. L. 1958. The determination and excretion of polyhydroxy (catecholic) phenolic acids in urine. J. Pharm. Pharmacol. 10:157-161.
- Trevett, M. E. and E. Austin. 1959. A note of the control of northern nutgrass in snap bean. Proc. NEWCC 13:494-495.
- Tripathi, R. L. 1964. A preliminary note on the beneficial lepidopterous larva feeding on nutgrass (Cyperus rotundus) in jute fields. Jute Bull. 26:255.
- Tsukamoto, H., H. Yoshimura, and T. Watabe. 1964. Metabolism of drugs. XLIX. The study of selective demethylation of 4-acetamino- and 4-nitro-veratroles in vivo. Biochem. Pharmacol. 13:1499-1505.
- Tumbleson, M. E. and T. Kommedahl. 1961. Reproductive potential of Cyperus esculentus by tubers. Weeds 9:646-653.
- Tumbleson, M. E. and T. Kommedahl. 1963. Factors effecting dormancy in tubers of Cyperus esculentus. Bot. Gaz. 133: 186-190.
- Vanden Born, W. H. 1961. Response of Tartary buckwheat growing in wheat to treatment with standard and low volatile esters of 2,4-D singly or in combination, Banvel-D, Banvel-T and Carbyne. Res. Rep. Natl. Weed Comm., Western Canada Sec. 8: 93-94.
- Vanden Born, W. H. and F. T. Chang. 1967. Translocation and persistence of dicamba and picloram in Canada thistle. Abstr. Meeting Weed Soc. Amer. 40-41.

- Veatch, C. 1958. Control of nutgrass (Cyperus esculentus) in corn with DNBP. Proc. NEWCC 12:200.
- Vengris, J. 1963. Nutgrass control in field corn. Proc. NEWCC 17:310.
- Vieitez, E. 1961. Accion de algunos herbicidas sobre la brotadura y errecimiento de les tubercules del Cyperus esculentus (The effect of some herbicides on the sprouting and growth of tubers of C. esculentus). An. Edef., Fisiol. Veg. 20:579-625.
- Weintraub, R. L., J. W. Brown, M. Fields, and J. Rohan. 1952. Metabolism of 2,4-dichlorophenoxyacetic acid. I. $C^{14}O_2$ production by bean plants treated with labelled 2,4-dichlorophenoxyacetic acid. Plant Physiol. 27:293-301.
- Wilcox, M. 1966. Separation of derivatives of 3,6-dichloro-o-anisic and 3,6-dichlorosalicylic acids and their acetic homologs by gas chromatography. Anal. Biochem. 16:253-259.
- Wilcox, M. 1967. Gas chromatography of alkyl ether derivatives of p-hydroxybenzoate esters. J. Pharm. Sci., in press.
- Wilcox, M., D. E. Moreland, and G. C. Klingman. 1963. Aryl hydroxylation of phenoxyacetic acids by excised roots. Physiol. Plantarum 16:565-571.
- Williams, R. T. 1949. Detoxication mechanisms. p. 95. Chapman and Hall, Ltd., London.
- Worsham, A. D., G. C. Klingman, and R. P. Upchurch. 1964. Nutgrass Cyperus rotundus and Cyperus esculentus control. N. C. State Agr. Coll. Ext. Circular 452.
- Worsham, A. D. and F. L. Selman. 1966. Special weed problems project, nutsedge. SWC Res. Rep. 211.

APPENDIX

Air temperatures, soil temperature, and rainfall during 1965 and 1966

Month	Air 5 ft above		Soil at		Rain- fall (in)
	soil surface		4 in depth		
	Max	Min	Max	Min	
1965					
January	69.6	43.7	62.0	55.3	2.51
February	72.5	48.4	65.8	57.5	5.80
March	75.7	52.5	70.9	62.5	4.82
April	84.4	59.4	83.6	74.4	1.78
May	89.9	62.0	90.8	80.0	2.21
June	87.4	67.6	87.8	79.5	15.74
July	89.2	70.1	89.9	81.5	10.86
August	91.0	71.3	90.7	82.1	7.62
September	88.1	71.3	88.1	81.1	5.16
October	81.5	60.7	79.8	73.3	1.57
November	76.2	53.7	71.1	66.0	1.55
December	70.1	45.2	61.5	56.0	4.41
1966					
January	64.6	43.4	59.4	54.2	3.58
February	68.8	46.2	60.6	54.5	6.18
March	74.0	48.8	70.0	61.1	1.93

Month	Air 5 ft above		Soil at		Rain- fall (in)
	soil surface		4 in depth		
	Max	Min	Max	Min	
April	81.1	55.5	79.4	69.7	1.85
May	84.8	65.5	83.5	75.9	8.92
June	86.5	67.4	87.9	80.2	7.26
July	91.5	71.9	91.9	83.0	4.46
August	90.4	70.8	90.5	81.9	5.11
September	87.8	68.5	86.3	79.9	12.25
October	82.3	63.1	79.8	74.7	1.44
November	74.6	49.5	69.2	64.0	0.59
December	68.5	43.9	60.3	55.9	1.13

BIOGRAPHICAL SKETCH

The writer is the son of Mahadev Roy and Amiya Roy and was born in Burdwan, West Bengal, India, on August 1, 1935. He attended the Municipal High English School, Burdwan, and passed the Matriculation Examination in 1950 from the University of Calcutta. The University of Calcutta conferred upon him the Bachelor of Science degree in 1955. In July, 1955, he entered the Banaras Hindu University and received the degree of Bachelor of Science in Agriculture in 1958 and was placed in first class.

He worked as an Agricultural Extension Officer under the Directorate of Agriculture, Government of West Bengal, from September, 1958, through December, 1962. In January, 1963, he started his graduate work in the University of Florida and received his Master of Science in Agriculture with major in Agronomy in June, 1964. He continued his graduate studies in the University of Florida for the Doctor of Philosophy degree.

He was elected as a member in the Phi Sigma society for the Biological Sciences.

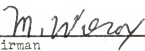
This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1967


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Supervisory Committee:


Chairman



